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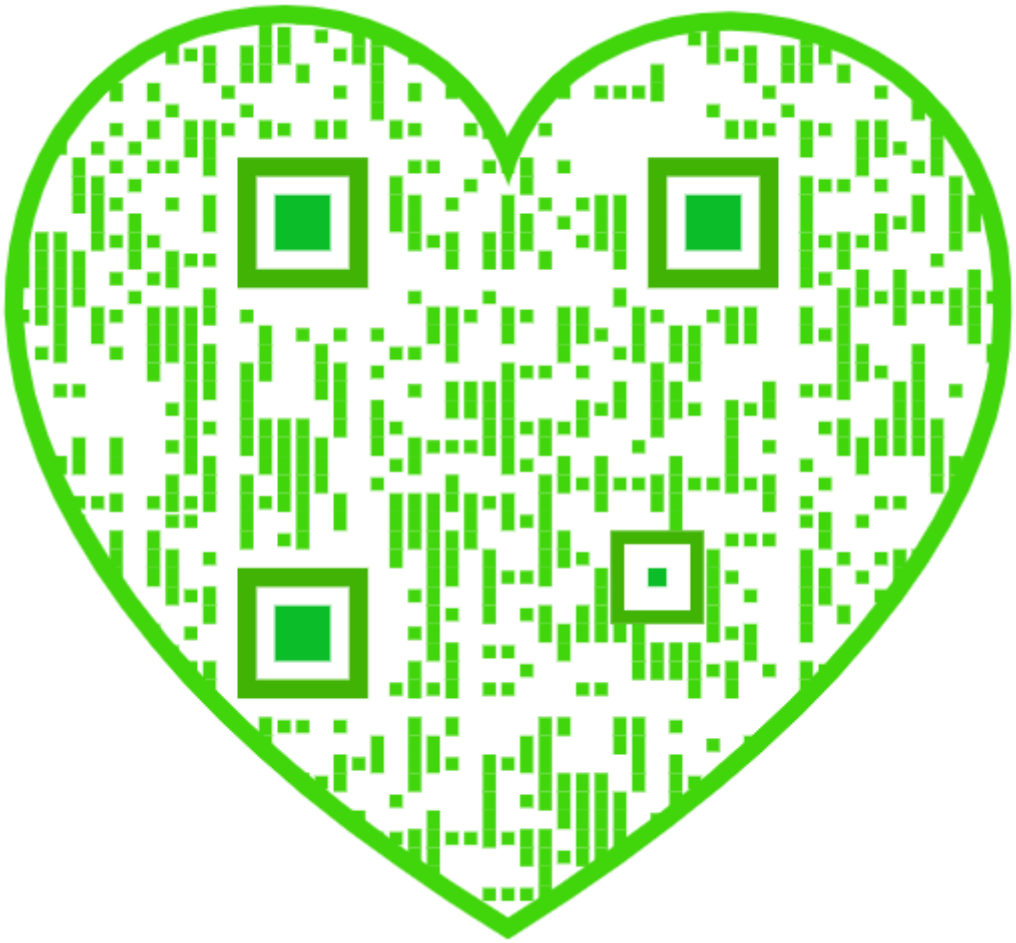
PRACTICAL BOTANY



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A COURSE OF PRACTICAL INSTRUCTION

IN

BOTANY

A

COURSE OF PRACTICAL INSTRUCTION

IN

BOTANY

BY

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WITH A PREFACE TO PART I. BY

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PART II.

BRYOPHYTA—THALLOPHYTA

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PREFACE.

In the preface to the *Course of Practical Instruction in Botany*, Part I., which was published in 1885, Mr. Dyer intimated that it was intended that a second part, comprising instructions for the practical treatment of types of organisms lower in the scale of vegetation, should follow the publication of Part I. After a delay of over two years, which has however been unavoidable, this second part is now completed, and may, together with the first part, serve to indicate to students the lines on which the study of the vegetable kingdom may be approached through the detailed observation of a few selected types.

Here, as in Part I., the selection of the types has been influenced, partly by the commonness and easily-recognised characteristics of the plants chosen, partly by their special adaptation to serve as typical representatives of the groups to which they belong; and it is

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hoped that the brief general description prefixed to the study of each type will suffice for the identification of the plant named, even by those students who work without the assistance of a qualified teacher.

I am myself responsible for the whole of this second part; but I wish to acknowledge help from many friends, and specially the constant assistance and criticism of Dr. Vines, who has revised all the proofs. Also I have received suggestions from Dr. Scott in connexion with the chapters on the Seaweeds, and from Professor H. Marshall Ward, who has kindly revised the chapters on the Fungi.

F. O. Bower.

Glasgow, *June*, 1887.

CONTENTS.

PRACTICAL DIRECTIONS FOR THE STUDY OF THE SELECTED TYPES.

BRYOPHYTA.

A. MUSCI. PAGE

Polytrichum commune 1

Sphagnum 16

B. HEPATICÆ.

Marchantia polymorpha 19

THALLOPHYTA—ALGÆ.

FLORIDEÆ.

Polysiphonia fastigiata 35

PHÆOPHYCEÆ.

Fucus serratus 47

Laminaria digitata 62

CONFERVOIDEÆ.

Coleochæte scutata 65

Œdogonium 67

Ulothrix zonata 70

SIPHONEÆ.

Vaucheria sessilis 74

VOLVOCINEÆ.

Volvox globator 82

Pleurococcus vulgaris 84

Hydrodictyon utriculatum 85

CONJUGATÆ. PAGE
Spirogyra 87
Desmidieæ 91
Diatomaceæ 93
CYANOPHYCEÆ.
Nostoc 95
Oscillatoria 96
Glœocapsa 96

THALLOPHYTA—FUNGI.

BASIDIOMYCETES.
Agaricus campestris 97
ÆCIDIOMYCETES.
Puccinia graminis 105
ASCOMYCETES.
A. DISCOMYCETES.
Peziza 113
B. LICHENS.
Parmelia parietina 115
C. PYRENOMYCETES.
Claviceps purpurea 123
D. CLEISTOCARPOUS ASCOMYCETES.
Eurotium Aspergillus glaucus 126
PERONOSPOREÆ.
Pythium de Baryanum 133
Cystopus candidus 137
MUCORINEÆ.
Mucor Mucedo 139
Sporodinia grandis 142

PRACTICAL BOTANY.

PART II.

BRYOPHYTA.

A.—MUSCI.

POLYTRICHUM COMMUNE, *L.*

A.—GENERAL EXTERNAL CHARACTERS.

I. Observe in well-grown specimens of this Moss taken in spring or early summer—

1. The erect **stem,**[1] which may attain a considerable length, branching but rarely:

2. The **leaves,**[1] of relatively small size, and simple form; their arrangement is on a complicated plan (see below): at the base of the stem note—

3. A dense mat of **rhizoids** of brown colour.

At the apex of some specimens will be found merely a bud, composed of young leaves of the vegetative type; other specimens will bear at their apex—

4. **Perigonia,** or **perichætia;** cup-like rosettes of leaves, which assume a bright reddish or orange colour,

[1] Though the terms "stem" and "leaf" are used here, it must be distinctly borne in mind that the members thus named, being parts of the **oophore generation** are **not homologous** with, but at most **only analogous** to the stem and leaf in vascular plants, which are parts of the **sporophore generation.**

and protect the **antheridia**; other specimens again may bear at their apex—

5. The mature **sporogonium** or spore-capsule, of which the head or **theca** is supported on a long stalk, or **seta.** Note in specimens which are not too ripe—

a. The **calyptra,** a dry fibrous hood, covering the apex of the sporogonium: beneath this is—

b. The lid-like **operculum** with its terminal beak: this lid may be easily detached, disclosing—

c. The thin membranous, and transitory **epiphragm.**

d. The **peristome,** a fringe of fine fibres, which by their hygroscopic movements assist in scattering the **spores.**

e. At the base of the theca observe a swelling called the **apophysis.**

f. By carefully removing the leaves from the apex of a plant bearing a sporogonium, it may be seen that the base of the seta is enveloped by a closely fitting sheath, the veil or **vaginula,** the origin of which will be explained later (page 9).

Observation of the external characters of a simpler Moss, *e.g. Funaria hygrometrica,* will give in the main similar results to those above described for the larger *Polytrichum.*

B.—MICROSCOPIC INVESTIGATION.

Oophore Generation.

II. Cut transverse sections of a mature stem of *Polytrichum:* mount some in glycerine, others in Schulze's solution or in iodine solution: examine them first under a low power, and observe in those mounted in glycerine—

1. The outline of the section, which is usually more or less clearly triangular.

2. The dense reddish-brown band of peripheral **sclerenchyma,** which passes over gradually into—

3. A broad, thinner-walled band of tissue, which may be termed the **cortex:** this finally surrounds—

4. A central mass of firm, yellow-walled tissue.

Examine these several tissues in detail, under a high power, and observe that—

1. At the extreme outer limit is a thin **cuticle,** with small and irregular outgrowths: there is no clearly defined epidermal layer.

2. The peripheral sclerenchyma consists of cells with clearly stratified red walls, which are of such thickness as almost to obliterate the cell-cavity.

3. The broad band of tissue of the cortex (3, above) has relatively thin, yellowish or colourless walls, and protoplasmic contents with starch granules, and globules of oil (compare sections treated with iodine).

4. The central mass of tissue (which may be compared to a **vascular bundle**) is not sharply limited from the cortex: it consists of—

a. A peripheral, small-celled, and thin-walled portion, the walls of which do not stain blue, but light yellow with Schulze's solution.

b. A central, thick-walled part, without cell-contents: the thick walls stain dark brown with Schulze's solution: the elements are often divided by delicate septa, which are not stained by Schulze's solution. This tissue may be compared to the **xylem** of true vascular plants.

Here and there small groups of tissues similar to the above may be seen in the cortex; these are the strands which enter the stem from the leaves, and pass inwards towards the central bundle.

III. Cut median longitudinal sections of the mature stem of *Polytrichum:* mount as before, and note that the peripheral sclerenchyma consists of elongated prosenchymatous elements, while the cells of the massive cortex are of a parenchymatous form. The elements of the central strand are elongated, and their lateral walls smooth, without pits: they are separated one from another by thin oblique septa. Observe that where the median plane of a leaf has been cut through longitudinally, a strand of tissues similar to those constituting the central strand may be traced, passing obliquely through the cortex towards the central strand.

By cutting transverse and longitudinal sections of the apex of the stem of *Polytrichum*, or *Funaria*, it may be ascertained that there is in each case **a single apical cell** of tetrahedral form; that segments are cut off successively from the three sides; and that one leaf originates from each segment: in the transverse sections it may, however, be seen that in both plants the angle of divergence between the successive leaves (and similarly between the successive segments) is larger than one-third: thus the leaves form three parastichies, and this will account for the apparent complexity of their arrangement in these plants. In *Fissidens* the arrangement is in two longitudinal rows, or orthostichies, and there is a bilateral wedge-shaped apical cell. In *Fontinalis* there is a tetrahedral apical cell, but the divergence of both segments and leaves is one-third.

IV. Strip off a few mature leaves: mount one of them in water, with the upper surface uppermost, and observe under a low power that the narrow, linear upper portion is marked on its upper surface by

longitudinal striæ (the **lamellæ**), and has a minutely serrated margin: the basal portion of the leaf, which is closely applied to the stem, is broad, but thin and membranous, and is not marked by longitudinal striæ.

V. Cut transverse sections of leaves: this may easily be done by holding the terminal bud of a mature plant between pieces of pith, or by embedding in paraffin, and then cutting transverse sections of the whole bud. Mount all the sections as before, and examine first with a low power. Neglecting the almost circular transverse sections of the stem, recognise—

1. Those transverse sections which have passed through the sheathing basal portions of the leaves: these may be readily distinguished by their broad lateral wings, only one layer of cells in thickness.

2. Those which have been taken from the upper part of the leaf: these may be distinguished by their more bulky appearance.

Having recognised these sections, put on a high power and examine them in detail:—

1. In the section of the sheathing base of the leaf observe—

a. The two lateral wings, consisting of a single layer of cells, with thickened outer walls, and but little chlorophyll.

b. The more bulky central portion consisting of—

i. An irregular layer of superficial cells (**epidermis ?**) with thickened outer walls, covering both upper and lower surface: beneath these are—

ii. Bands of **sclerenchyma,** in which the lumen is almost obliterated.

iii. Within these lies a "vascular bundle" consisting

of elements essentially similar to those composing the central bundle of the stem.

2. In the sections of the upper part of the leaf note that the arrangement of the tissues is for the most part similar to that in the above sections, but rather more bulky, while opposite each of the cells at the upper surface is seen to be attached a series of three to five chlorophyll-containing cells, which represent transverse sections of those longitudinal plates or **lamellæ** above observed on the upper surface of the leaf, under a low power. It is now obvious that these chlorophyll-containing lamellæ are separate laterally from one another: they constitute the chief assimilating tissue of the plant.

For comparison with the above, a Moss of simpler type may be taken, *e.g. Funaria hygrometrica.* As before there is an erect stem, with a mat of brown rhizoids at its base : it bears a number of leaves of somewhat variable form, more or less widely ovato-lanceolate.

Mount a single leaf in water and examine under a low power : note the clearly marked midrib, terminating in the acuminate apex, the thin lateral portions, consisting of only a single layer of cells containing chlorophyll, and bounded by an entire margin.

Cut transverse sections of the stem, and mount in weak glycerine or glycerine jelly : on examination under a low power it will be seen that the stem is of much simpler structure than that of *Polytrichum :* the peripheral tissues have brown walls, but they are not thickened to any marked degree : at the centre is a strand of thin-walled, small-celled tissue : the peripheral tissues usually contain chlorophyll.

Sexual Organs.

VI. Take a mature antheridium-bearing axis of *Polytrichum,* and dissect it with needles in a watch-

glass, keeping all the parts detached. Examine them carefully with a lens, and observe the following categories of organs—

1. The **perigonial leaves,** which are widened laterally into very broad membranous wings, with a clearly defined, central midrib.

2. The white, club-shaped **antheridia.**

3. The **paraphyses,** which will often be found associated with the antheridia: some of them are simply filamentous, others are more or less clearly spathulate.

VII. Cut median longitudinal sections of a male axis: mount in weak glycerine, and with a low power recognise the several organs above described, and their relative positions: note especially the **antheridia** in the axils of the perigonial leaves. Observe under a high power the structure of a single antheridium: it consists of a short **stalk,** and a club-shaped body, composed of (i.) a **wall** a single layer of cells in thickness, and (ii.) a central mass of cells of more or less clearly cubical form: these are the **mother-cells of the antherozoids.**

VIII. Take fresh antheridium-bearing specimens of *Polytrichum* after some days of dry weather (or keep them rather dry for some days, carefully preventing any access of water from above): squeeze one of them between the finger and thumb: the antheridia will thus be easily forced from their position, and may be mounted in water. If they were properly mature, it may then be seen that on contact with water the antheridia burst, and the mother-cells escape, aggregated in a mass. In each cell of this mass a spiral filament

may be seen, in active movement: it may be seen to escape ultimately, owing to mucilaginous swelling of the wall of the mother-cell, as a free **antherozoid** of spiral form, having two cilia.

Antheridia may be obtained on *Funaria hygrometrica* at almost any period of the year: they are borne on special branches as in *Polytrichum*, but are surrounded by a less conspicuous perigonium: these axes are usually shorter than those which bear the female organs.

IX. Take a sod of *Funaria*[1] with no sporogonia as yet visible upon it, but which bears antheridia: these will be situated at the apices of the shorter axes: many of the longer axes will appear to be terminated by ordinary vegetative leaves, and it is on these axes that the archegonia may be found.

From such buds, after hardening in alcohol, cut median longitudinal sections: if not transparent enough treat with dilute potash solution, and mount in weak glycerine: subsequently the sections may be transferred to glycerine jelly. Examine first with a low power, when the usual arrangement of axis and leaves may be observed; between the youngest leaves an archegonium (or several) may sometimes be detected. If mature, it will be seen to be a flask-shaped organ, seated on a short massive **stalk**: it consists of—

1. An elongated **neck**, more or less contorted, com-

[1] Since *Funaria hygrometrica* produces sporogonia at all times of year, and is very common, while *Polytrichum commune* is reproduced sexually only in the spring and early summer, it will be convenient in most cases to use the former in examining the archegonia. In either case, however, it is a matter of some experience and expenditure of time to get a good series of preparations illustrating the development and structure of the archegonium, and the early stages of the production of the sporogonium.

posed of a single layer of cells arranged in four to six rows: these surround a central **canal,** which is filled with mucilage at the time of fertilisation, but before maturity there may be seen within it a series of **canal cells.**

2. A lower, enlarged **ventral portion,** consisting of two layers of cells, which constitute the **wall,** and inclose a central space, in which may be seen the naked spherical **oosphere** or **ovum,** and above it (up to the period of maturity) the smaller **ventral canal cell.**

Similar sections, similarly prepared from rather older specimens, may show as the first results of fertilisation that the neck of the archegonium turns brown and withers, while the wall of the ventral portion and the stalk show considerable increase in bulk, and frequent cell-divisions. Meanwhile growth and cell-division take place also in the fertilised ovum, resulting ultimately in the development of the sporogonium: the growth of the wall of the archegonium keeps pace for some time with that of the young sporogonium, completely inclosing it, but as it increases towards maturity, the wall of the archegonium is ruptured transversely about half-way up: the apical part is carried upward by the growing sporogonium, as the **calyptra** which covers its apex, while the lower portion remains as an investment of the base of the seta, and is called the **veil** or **vaginula.**

Note especially in young sporogonia the two-sided, wedge-shaped apical cell, with segments cut off from either side.

Sporophore Generation.

The Sporogonium.

X. Having noted the external characters of the sporogonium of *Polytrichum*, as above described, cut transverse sections of the mature seta: mount in

glycerine or glycerine jelly. Being a cylindrical organ the transverse section is circular. Note—

1. The superficial layer of cells with a definite cuticle and thick yellow walls.

2. A band of brown **sclerenchyma,** which graduates internally into—

3. A thin-walled **parenchyma** with large intercellular spaces, and containing chlorophyll.

4. Centrally is a strand of denser tissue without intercellular spaces.

XI. Cut median longitudinal sections of the base of the seta, which is inserted on the apex of the Moss-plant or oophore: mount as before, and note in the upper part of the seta the superficial layer, brown sclerenchyma, thin-walled parenchyma, and central strand, as above described. Following the seta down to the base, it will be seen that the cuticle and brown sclerenchyma stop short, and are replaced by thin-walled parenchyma with plentiful protoplasm; this tissue of the sporophore is in close connexion with the inner surface of the **vaginula,** which belongs to the oophore generation. With this close physiological connexion of the sporophore and oophore in the Moss, compare the connexion by means of the foot in Ferns.

Similar sections may be prepared, with similar results, from plants of *Funaria*, but there will be greater difficulty in this case, owing to the smaller size of this Moss.

XII. Passing now to the apex of the sporogonium of *Polytrichum* remove the **calyptra:** mount it in water or weak glycerine, and examine under a low power. It consists of dry, often branched, hypha-like filaments, loosely matted together: the neck of the

archegonium may often be recognised at its extreme apex.

The calyptra of *Funaria* may be treated in a similar way: here the brown neck of the archegonium is clearly seen, while the body of the calyptra consists of a continuous tissue, a single layer of cells in thickness.

XIII. It will be found convenient to take first the capsule of one of the simpler Mosses, *e.g. Funaria*, and subsequently to proceed to a more complicated example, *e.g. Polytrichum*.

After noting the oval form, and the obliquely placed **operculum,** embed capsules of *Funaria*, which have been hardened in alcohol, in paraffin: and cut median longitudinal sections: mount in glycerine or in glycerine jelly, and examine first with a low power.

N.B. It will be well to select young capsules of such age that the peristome (seen through the operculum) shall show a pale yellow tinge: later it assumes a dark yellow or orange colour, and in such cases the spores would be almost mature: the structure of the whole capsule would accordingly be more difficult to understand than in the younger specimens.

Observe—

1. The **seta,** which widens out gradually into—

2. The **theca** or **capsule,** without any clearly marked basal swelling, or apophysis: at the apex of the **theca** observe that the section has traversed—

3. The **operculum** or dome-like lid: beneath this is—

4. The yellow or orange **peristome:** in the lower part of the capsule note—

5. The outer **wall** consisting of a clearly marked **epidermis,** and beneath it some three or four layers of thin-walled parenchyma.

6. The **air-space,** traversed obliquely by filaments of thin-walled cells, suspending a central mass consisting of—

7. The **spore-sac,** which will be seen to be composed of two or three layers of thin-walled cells.

8. A layer of **spore-mother cells,** recognised by their dense protoplasmic contents.

9. The central mass of thin-walled tissue of the **columella,** which extends upwards into the concave operculum.

By careful observation of longitudinal sections under a high power the following points may be verified :—

1. That **stomata** occur in the epidermis towards the base of the sporogonium.

This may be confirmed by cutting tangential sections from the base of the sporogonium, in which the stomata, with their two guard-cells, may be clearly seen in surface view.

2. That immediately above the upper limit of the air-space there is a band of brick-shaped cells, elongated transversely, and with pitted walls : these are not derived from the epidermis but from the subjacent layers, and serve to connect the peristome with the outer wall of the theca. Closely above this band the tissues are again more delicate, and it is here that the **rupture of the capsule** takes place, by which the operculum is set free. Above this more delicate zone is—

3. The slightly projecting lower lip of the operculum, at which point the epidermal cells are thickened, and of peculiar form, constituting the **annulus.** Beneath the operculum, which consists of the superficial layer of epidermis together with two or three layers of thin-walled tissue, lies—

4. A layer of cells with peculiar yellow or brown thickening of the walls: this gives rise to the **peristome.**

Returning to the **spore-forming layer,** it may be verified under a high power—

i. That it consists at first of a **single** layer of cells (the **archesporium**): to ascertain this for certain it may be necessary to cut sections from sporogonia of various ages.

ii. That the cells of this layer divide repeatedly; and ultimately, by division of each of the resulting spore-mother cells into four, the **spores** are produced.

Mount some mature spores in water: they will be seen to be of spherical form, with smooth walls, and granular, oily contents.

XIV. In order better to understand the structure and origin of the **peristome,** cut off transversely the whole orange-coloured tip of a sporogonium of *Funaria,* and mount it with the apex uppermost in weak glycerine: observe under a low power—

i. The contorted brown **teeth** of the peristome, sixteen in number: these are easily seen through—

ii. The more transparent **operculum,** which covers them: by focusing carefully downwards—

iii. The much thickened cells of the **annulus** may also be distinctly seen.

XV. Cut thin transverse sections of the operculum of a still unripe sporogonium: in those which pass immediately above the annulus, observe at the periphery—

a. The thickened **epidermis,** and beneath it two or three layers of thin-walled cells: these together with the epidermis constitute the **operculum:** then follows—

b. A layer of cells with peculiar thickening bands on the inner and outer walls: these bands separate, by rupture of the thinner parts of the walls, as the **inner** and **outer peristome,** which thus consists when mature of ribbands of cell-wall, and not of complete cells.

The above points may with advantage be confirmed by observations on mature sporogonia.

For comparison with the sections above described, transverse and longitudinal sections may be cut from young sporogonia of *Funaria* of various ages, and the development of this peculiar organ may thus be traced. For details of description see the text-books; especially Goebel's *Outlines*, Eng. Ed., p. 186.

XVI. Longitudinal and transverse sections may also be made from the sporogonium of *Polytrichum commune,* and this may be treated as above directed. The chief differences from *Funaria* will be as follows—

1. The clearly marked apophysis.

2. The presence of **two concentric air-spaces,** with the spore-sac between them.

3. The more bulky operculum.

4. The proportionately smaller peristome, consisting in this case of bundles of prosenchymatous cells.

5. The presence of an **epiphragm**, below the operculum, and connecting the teeth of the peristome temporarily together.

6. The less clearly marked annulus.

XVII. Scatter spores from the ripe sporogonium of *Funaria* or *Polytrichum* over moist soil, and keep them at a moderately high temperature, under a bell-glass, for a few days. The surface of the soil will soon be seen

to be overgrown by numerous fine green filaments. Having carefully removed some of these with a needle, and having washed the soil from them, mount them in water, and examine them under a high power. Note—

i. The dark-coloured **exospore,** which may be found still attached to the filaments after they have attained a considerable length.

ii. The fine filamentous **protonema** resulting from out-growth of the **endospore:** observe especially the **septa,** which are often oblique; the **branches,** usually arising immediately below a septum: the various development of these branches, either—

a. As relatively thin filaments with brown cell-walls, and no chlorophyll: these are the **rhizoids,** and they penetrate the soil.

b. As relatively thick filaments, with colourless cell-walls, and chlorophyll: these constitute the true **protonema.**

c. As solid buds, which are usually situated at the base of one of the branches such as *a* or *b*: in these solid buds of various ages may be traced the successive stages of development of the **Moss-plant,** which is thus produced as a lateral bud on the protonema.

Cultures of protonema, showing all the most important characters above noted, may be obtained at any time of year by cutting fine sods of *Funaria*, inverting them under a bell-glass, and growing them in moist air and at a moderate temperature for two or three weeks. In the case of *Funaria* the protonema may under these circumstances be induced to form terminal unicellular **gemmæ,** which are easily detached from the parent protonema, and by germinating reproduce the plant in a vegetative manner.

It will also be found possible, by culture of detached leaves

and portions of the stem of the Moss-plant on moist soil, and under other favourable conditions, to induce a formation of protonemal filaments by direct outgrowth of cells of those parts.

SPHAGNUM (*the Bog-Moss*).

XVIII. Take a plant of any native species of *Sphagnum*, and observe with the naked eye, or with a pocket lens—

1. The brown **stem** on which are inserted—

2. **Leaves** of simple form.

3. The **lateral branches**, with fasciculate branches of higher order, which in their turn bear leaves: these branches assume two distinct forms—

a. Stronger branches, of larger size, which have their apices directed upwards.

b. Weaker branches, which are deflexed, and are usually found in close apposition to the main axis.

N.B. Owing to the main axis being thus closely covered by the weaker deflexed branches, the leaves borne by the main axis may escape observation: to prevent this, the branches should be entirely removed, and the leaves will then be easily seen in their normal position: it may further be noted on observing the leaves carefully that the lateral branches are not axillary, but are inserted **alongside of a leaf**, and that a fascicle of branches is associated thus with **every fourth leaf** of the main axis.

Nothing comparable to the protonema of other Mosses is to be found in *Sphagnum*, except under certain conditions of germination of the spore.

XIX. Cut transverse sections of the main axis of a plant of *Sphagnum*, and mount in weak glycerine or glycerine jelly: examine under a low power, and observe—

1. At the periphery of the section two or three irregular layers of cells with thin walls, and no cell-contents: lying internally to these is—

2. A dense brown band of tissue which merges gradually into—

3. A massive central pith, of comparatively large cells, with thin cell-walls and but little protoplasm.

Examine the peripheral tissues under a high power: round holes will be observed in the cell-walls as seen in surface view,

while accurate observation at the points where the longitudinal walls have been cut through will show that these circular markings are actually **open pores**, by means of which the cavities of the cells of this tissue communicate one with another, and are ultimately in open communication with the medium in which the plant grows. This tissue serves as a capillary system, by means of which water is supplied to the inner tissues of the stem.

XX. Strip off one or two leaves : mount in water, or very weak glycerine, and examine under a low power: note—

1. The simple form of the leaf, and its entire margin.

2. The absence of any midrib.

3. The simple structure, the leaf consisting only of a single layer of cells, amongst which two types may be recognised under a high power, viz.—

a. Large colourless cells, the walls of which are marked by annular or spiral bands, and showing here and there round open pores similar to those already observed in the superficial layers of the stem: these cells have no cell-contents.

b. Smaller cells of narrow form, easily recognised by their containing green chlorophyll granules embedded in colourless protoplasm : these cells, being attached by their ends one to another, together form a network, the meshes of which are occupied by the large colourless cells, *a.*

XXI. It may be found difficult to obtain material for the practical study of the sexual organs of *Sphagnum*, and it will accordingly suffice here to refer to the descriptions given of them in text-books (*e.g.* Goebel, *Outlines of Classification*, p. 183, &c.).

If specimens of the sporogonia be at hand, note with the naked eye—

1. The spherical form of the sporogonium.

2. Its position, seated on the end of a more or less elongated stalk—the **pseudopodium** : this must not be confounded with the seta of other Mosses ; as sections will show, the true seta in *Sphagnum* is short, and the pseudopodium is merely a prolongation of the axis of the oophore generation.

3. The **calyptra**, which may be, according to age, a more or less complete covering of the sporogonium, and is derived from the wall of the archegonium.

4. Where the calyptra has been broken away or removed, the circular lid-like **operculum** may be seen at the apex.

Cut longitudinal sections of the sporogonium: mount in glycerine or glycerine-jelly, and, selecting those which are median, examine under a low power—

1. The outline of the **sporogonium,** with its rounded head, and short **seta,** enlarged at its base into a broad foot.

2. The **pseudopodium,** which is in close physiological connexion with the foot of the sporogonium, and is continuous upwards into—

4. The **calyptra,** which may be seen, according to age, more or less completely enveloping the sporogonium.

Turning to the internal structure of the sporogonium, observe—

1. The central, massive **columella,** consisting of thin-walled cells: it is rounded off at its apex.

2. The **spore-forming layer,** which appears semilunar in section, being in reality of a bell-shape: according to the age of the sporogonium, spores of various stages of development may be seen composing the spore-forming layer.

3. Outside this is the **wall** of the sporogonium, consisting of some three or four layers of thin-walled cells, enveloped by a single layer of cells with thick brown walls. In this layer note towards the apex two points where the cells are smaller: here the section has traversed the circular line of rupture of the operculum. There is no peristome.

B.—HEPATICÆ.

MARCHANTIA POLYMORPHA, *L.*

A.—GENERAL EXTERNAL CHARACTERS.

I. Taking a fresh growing sod of *Marchantia* observe the following external characters with the naked eye, or by help of a pocket lens:—

1. The **flattened form,** sinuous margin, and prostrate position of the branched, green thallus.

2. Its dull, dark green upper surface, marked by diamond-shaped areas, and in the middle of each of these a dot, which is a single **stoma** (see below).

3. Projecting from the upper surface may also be seen in most cases small **circular cups,** with a finely crenate margin, in which may be seen numerous dark green flattened bodies, the **gemmæ:** these may be easily detached by slight mechanical disturbance.

4. Note the **organic apex** of the thallus, situated at the base of a terminal depression (compare the prothallus of Ferns): also that the branching is **dichotomous,** though the ultimate development of the originally similar branches is unequal, so that the result is a **sympodium.**

5. In some cases the branches of the thallus may have assumed peculiar forms, together with an **erect position:** these are the branches which bear the sexual organs, and two different types may be easily recognised as borne upon different individual plants, viz.—

a. Branches with a relatively thin stalk, bearing a terminal disk with crenate margin, and having numerous dot-like markings on the upper surface: these are the **male branches,** having the **antheridia** on their upper surface.

b. Branches, also with thin stalks, bearing a terminal star about $\frac{1}{4}$ inch to $\frac{1}{2}$ inch in diameter: these are the **female branches,** which produce the **archegonia** on their under surface, and ultimately the **sporogonia** and **spores.**

II. Remove a thallus carefully from the soil, and wash with water, taking care not to injure it, and examine the organs on its lower surface with a pocket lens: note especially—

1. The numerous **rhizoids** or root-hairs, attached chiefly to the central midrib.

2. The **amphigastria,** white or purple lamellæ attached to the lower surface of the thallus, and most clearly seen in the regions near the apex, where they are closely aggregated so as to protect the young tissue. These may be compared with the "leaves" of the Mosses (cf. note, page 1).

There will frequently be found growing in positions suitable for *Marchantia*, and often associated with it, another Liverwort called *Lunularia*, which is similar in general contour, but is usually of a lighter green colour: it may readily be distinguished

by the form of the cups containing the gemmæ, these being, as the name *Lunularia* implies, of a semilunar form. It is to be remarked further that *Lunularia* differs from *Marchantia* in certain structural details; and also in the fact that, though reproduction by gemmæ is most profuse, a sexual reproduction is not effected in this country. Thus it cannot be taken as a substitute for *Marchantia*, and the material must be carefully sorted before use.

For comparison with *Marchantia*, observations should also be made of other allied forms, *e.g. Riccia fluitans*, which is of simpler structure, and of much smaller size; it branches dichotomously, and the branches in this case often develop equally. If a fresh thallus of *Riccia* be examined under a low power, it will be seen to consist of green chlorophyll-containing cells, with numerous large intercellular spaces, and the apex will be seen to be depressed; on the under surface will be found small amphigastria.

A comparison should also be made with *Pellia*, in which the thallus is of a broad, frondose form, of simple structure.

Some species of *Jungermannia* should also be examined: here the shoot will be found to be dorsiventral, but of more complex external form than that of *Marchantia*; in addition to the amphigastria, there are also present lateral appendages or "leaves," which are thin, flattened expansions of green chlorophyll-containing tissue, inserted on the relatively thin axis.

B.—MICROSCOPIC OBSERVATIONS.

III. Cut transverse sections of the vegetative thallus of *Marchantia*, avoiding at first the cups bearing the gemmæ, and the sexual branches. It is easier to use material hardened in alcohol, and to embed it in paraffin, or hold it between pieces of pith; but if sections be cut from fresh green material the presence of chlorophyll will be found to be an advantage in distinguishing the tissues. Mount some sections in

weak glycerine, others in Schulze's solution, or in iodine solution: examine under a high power, and, starting from the upper surface, observe—

a. The **superficial layer,** or so-called "epidermis," consisting of a continuous layer of cells of small size which contain chlorophyll, as may be seen in preparations from fresh material: the continuity of the layer is broken here and there by the so-called "stomata": these however differ from the true stomata of the higher plants in the mode of their development. This layer may be seen to be attached to the lower lying tissues at points between the "stomata," and the lines of attachment thus cut through correspond to the limits of the diamond-shaped areas above noted.

b. Beneath the "stomata" are large areas, the **air-cavities,** in which are seen numerous round or oval cells, grouped in simple or branched series, and attached to the lower surface of the cavity: their cell-walls are thin, and consist of cellulose: these cells contain chlorophyll, and constitute the chief assimilating tissue of the plant.

c. Below this is a massive tissue, which constitutes the great bulk of the section: it consists of oval cells, with few intercellular spaces, if any at all: the walls are thin, and marked with shallow pits: the protoplasmic contents are scanty: in the cells nearer the upper surface there are often numerous starch-grains. Individual cells here and there in this tissue have peculiar mucilaginous, or highly refractive, yellowish or brown, oily contents.

Cut transverse sections from a fresh thallus, mount in water, and having noted cells containing the highly refractive bodies

above mentioned, treat with potash solution, and warm gently over a spirit-lamp: the bodies are not dissolved, but partially lose their highly refractive quality, without swelling. Treat other sections first with alcohol, and then with ether: wash from the ether with alcohol, and mount: the oil bodies will have been dissolved.

d. At the lower surface of the thallus may be seen attached organs of two kinds—

i. **Hairs,** or **rhizoids,** which are long and unicellular, and are inserted deeply in the tissues of the thallus: they often show dotted or peg-like ingrowths of the cell-wall of various form.

ii. The **amphigastria,** which may now be seen to be plates of tissue one layer of cells in thickness: their cell-walls are often coloured violet or brown.

Returning now to the **"stomata,"** note under a high power their structure as seen in a good transverse section: each will appear as consisting of tiers of small cells (four or more in depth), which surround a large central cavity.

IV. Cut tangential sections so as to strip off the so-called **"epidermis":** mount with the outer surface uppermost in weak glycerine: observe under a low power the diamond-shaped areas above described, and a single large "stoma" in the middle of each. Under a high power note—

1. That the cells of the "epidermis" contain chlorophyll.

2. That each "stoma" is bounded by four or five of the tiers of cells above described.

3. That these cells contain but little chlorophyll.

4. That on focusing downwards it becomes apparent

that the lowest cell of each tier projects into the cavity of the "stoma," so that the channel at that point presents a stellate appearance in surface view.

Sections should also be cut from the thallus of *Lunularia*, wherein it will be found that the stomata do not correspond to the above description, but are of a simpler type, and it is well to compare the structure in *Lunularia* with that in *Marchantia*.

V. Remove a number of the **rhizoids**: mount them in water or glycerine, and examine under a low power: two types of these elongated unbranched hairs may be recognised—

a. Those with smooth walls: these are the more numerous:

b. Those with peg-like projections of the wall into the cavity of the hair: these projections are arranged in a more or less clearly spiral manner, and they may not unfrequently be seen to be branched irregularly: the protoplasmic contents are very scanty.

VI. Avoiding, as before, both the cups with gemmæ, and the sexual branches, cut from material hardened in spirit fine median longitudinal sections of the thallus, so as to include the depressed apex: mount in glycerine, and examine under a high power.

In the older part of the section the structure of the thallus will be seen as above described: on approaching the apex, note that the air-cavities are successively smaller, and the chlorophyll-containing tissue gradually disappears. The thallus has a **blunt apex** covered by numerous amphigastria, which thus protect it. At the extreme organic apex may be seen a single wedge-shaped cell, which is, however, only one of a **series of initial cells** of like form, as may be ascertained on careful investigation by

means of transverse sections of the apex. In good median longitudinal sections of the apex, note also—

1. The origin of the amphigastria, as plate-like outgrowths on the ventral surface, immediately below the apex of the thallus.

2. The formation of the air-cavities: these appear to be formed by involution of the outer surface, and subsequent over-arching of the cavity thus formed; but the actual proof of this is a matter of some difficulty, and requires careful cutting of sections.

3. The development of the so-called "stomata" by division of certain cells by periclinal walls.

4. The origin of the chlorophyll-containing tissue, by budding of the cells forming the floor of the young air-cavity, and frequent subsequent branching.

Similar preparations may be made from *Lunularia* and *Fegatella* with similar results: in these it is more easy to trace the origin of the air-cavities. Note especially in these forms the simpler structure of the "stomata," and the peculiar mucilage-cells, which in *Fegatella* are associated in longitudinal series, and attain a large size.

Preparations should also be made to illustrate the structure of the apical meristem in other forms: thus, in *Metzgeria* there may be found a single two-sided wedge-shaped apical cell, in *Jungermannia* a three-sided, and in *Blasia* a four-sided apical cell. These examples will suffice to show that the type of apical structure is far from being uniform throughout the Liverworts.

Gemmæ.

VII. Remove from one of the cups on the upper surface of the fresh thallus of *Marchantia* some of the **gemmæ:** mount them in water, and note under a low power—

1. The flattened disk-like form of the gemma, presenting a nearly circular outline, with two lateral indentations, and a scar at the base where it was attached to the thallus which produced it.

2. The ordinary chlorophyll-parenchyma of which it is mainly composed.

3. Superficial hyaline cells, from which the rhizoids are subsequently derived.

4. The single cells containing oil-bodies.

It may further be observed that the gemma is in its peripheral part only one layer of cells in thickness, while the central part is a solid mass: also that the structure is alike on both sides of the gemma, *i.e.* that it does not as yet show any trace of a dorsiventral character.

VIII. Cut transverse sections of a thallus so as to pass through the middle of one of the **cups**: mount in very weak glycerine, or in water, and examine under a low power: note—

1. The two lips of the cup, which appear as outgrowths from the upper surface of the thallus, and show more or less clearly the same structure, especially in the lower part.

2. The numerous **gemmæ,** in various stages of development, which are attached to the base of the cup by unicellular stalks.

3. Unicellular outgrowths or hairs, with mucilaginous walls.

Trace under a high power the process of development of the gemma. It may be seen that the gemma originates as a unicellular papilla, which divides first into two cells: the lower remains quiescent as the unicellular stalk, while the upper cell grows and divides, ultimately giving rise to the mature gemma. Trace the succession of cell-divisions by comparison of gemmæ of various ages.

Removing some gemmæ from the cups, germinate them on clean moist sand under a bell-glass at a medium

temperature, and exposed to the light: on examining them after five or six days, they will be seen to be elongated transversely to their original axis of growth, the base of each of the lateral indentations serving as an organic apex: from the lower surface root-hairs have been formed, by simple outgrowth of single cells. After growth has been continued for a longer time the differentiation of tissues characteristic of the mature thallus, with "stomata" and air-cavities, becomes apparent on the upper surface, the thallus thus assuming a dorsiventral character.

The Male Branch (Antheridiophore).

IX. Having noted the general form of the male branch of the thallus, with its stalk and terminal disk, cut transverse sections of the stalk, mount in glycerine, and examine under a medium power. Observe—

1. The circular **outline** of the sections.

2. Two **involutions** of the margin, corresponding to two channels, which traverse the stalk longitudinally: due investigation will show that these are on the morphologically lower face of the stalk.

3. **Rhizoids** will be found traversing these channels longitudinally.

4. The tissues seen in these sections are not clearly differentiated.

X. Cut median vertical sections through the terminal disk: mount in weak glycerine, and examine first under a low power: observe—

1. The general **outline** of the section, and especially the attachment of the disk, and the flat upper surface.

2. The **amphigastria** and **rhizoids** attached to the lower surface.

3. The **cavities** in the tissue, of two sorts, both opening by narrow mouths on the upper surface—

a. **Air-cavities** fundamentally similar to those of the vegetative thallus, with a "stoma" above the centre of each, and with chlorophyll-containing cells as before.

b. **Flask-shaped cavities** each containing one **antheridium :** these also open by a narrow channel on the upper surface of the disk.

Look for a single ripe antheridium which has been cut through longitudinally: having found one, examine it in detail under a high power, and observe—

1. The short **stalk** by which it is attached to the base of the cavity.

2. The **wall** of the antheridium, consisting of a single layer of thin-walled cells.

3. The **mother-cells of the antherozoids** of cubical form, and small size, which together constitute a dense central mass.

Trace the channel of one of the flask-shaped cavities up to the surface of the thallus, and note that the structure of the pore is quite distinct and different from that of the "stoma."

Compare antheridia of various ages in sections cut from younger male branches: by such a comparison it may be ascertained that each antheridium arises from a single cell at the base of the flask-like cavity: trace the successive divisions which accompany the development of one such single cell into the mature antheridium.

Cut tangential sections from the upper surface of a male branch, and under a high power note—

a. The "stomata," of similar character to those on the vegetative thallus.

b. The pores or openings of the cavities in which the antheridia are seated. These pores are of about the same size as the "stomata," but differ from them in being often triangular, and in the number (nine) and arrangement of the surrounding cells.

The Female Branch (Archegoniophore).

XI. Remove the star-shaped head of a female branch which has attained a considerable length, and examine first the upper surface: with the naked eye or with a lens note the rounded arms, nine in number, and the diamond-shaped areas, each having a single central stoma.

Turn the head upside down, and observe on its lower surface—

1. The central attachment or **stalk.**

2. The radiating **arms,** usually nine in number.

3. The curtain-like **perichætia,** which alternate in position with the arms: the **archegonia** are enveloped by these, and if the branch be an old one—

4. The nearly spherical **sporogonia** may be observed protruding from them: if these be fully ripe, they may have burst, in which case a yellow flocculent mass may be seen protruding from them, consisting of the **spores** and **elaters.**

XII. Cut transverse sections of the stalk, and mount in glycerine: examine under a medium power, and compare with similar sections of the stalk of the male branch: the general arrangement of the parts is the same, the chief differences being in this case—

1. The quadrangular outline of the sections.

2. The presence of air-cavities on the morphologically upper surface, with "stomata," and small quantities of chlorophyll-containing parenchyma: in fact the structure of the female stalk corresponds more nearly than the male to that of the ordinary vegetative thallus, of which they may both be regarded as modifications.

XIII. It will be well to start the study of the female organs on a younger branch than that above described: select a female branch which has not yet grown more than a quarter of an inch in height,—cut rather thick transverse sections (*i.e.* in a horizontal plane) through the head of it, mount in glycerine, and examine under a low power: observe—

1. The central **stalk** cut through transversely, and presenting characters similar to those above described.

2. The **arms,** usually nine in number, radiating from that central point (it may be further noted that the largest gap between the arms is directly opposite the morphologically upper surface of the stalk, and that in this gap there is no group of archegonia):

3. The numerous **archegonia,** each of which presents a circular outline from this point of view: they are disposed in groups alternating in position with the arms, each group being surrounded by the perichætium.

Under a high power the following points may be ascertained—

a. That the most mature archegonia are those nearest the periphery, while those of each group are successively younger the nearer they are to the central stalk.

b. That each mature archegonium as seen in section

consists of a wall (one layer of cells in thickness) which surrounds and incloses a large, naked, nucleated **ovum.**

XIV. Cut vertical sections through a female branch of like age to the above: treat as before, and observe in median sections—

1. The **stalk,** on which is borne—
2. The terminal stellate **head.**
3. That the latter consists of tissues similar to those of the vegetative thallus: note especially—
   a. The superficial "epidermis," and "stomata."
   b. Air-cavities with chlorophyll-parenchyma.
   c. Large mucilage-cells.
4. The **archegonia,** which are flask-shaped bodies suspended in an inverted position: note in a mature archegonium—
   a. The long **neck,** consisting of many tiers of cells, which together form a single-layered cylinder surrounding the channel of the neck: when mature the neck will be seen to be open at its apex.
   b. The more distended lower ventral part of the archegonium, also surrounded by a single-layered wall, and attached by a short massive stalk to the receptacle.
   c. Pay special attention to the contents of the ventral portion: in the mature archegonium the cavity will be seen to be occupied by a single primordial cell—the **ovum** or **oosphere**—to which access is gained from without through the channel of the neck.

It is perhaps easier in these plants than in any others to trace the successive stages of development of the archegonium : this may be done by cutting sections, similar to those above described, from young female receptacles, and noting first those archegonia which are situated nearest to the stalk ; these should then be compared with those further removed from it, which will be found to represent successively older specimens : thus the several stages of development may be traced even in a single specimen, and they should be carefully compared with the description and figures given in the standard text-books (*e.g* Goebel, *Outlines of Classification*, Eng. Ed. pp. 150, 162). The attempt should further be made to observe the actual rupture of the neck of the archegonium, by preparing sections of the female receptacle, mounting dry ; then, having found a suitable archegonium, add a drop of water, when the canal cells and ventral canal cell may be seen to be suddenly extruded on rupture of the apex of the neck.

XV. Keep some specimens of *Marchantia*, having mature male receptacles, protected for some days from access of water from above : then place a drop of water on the upper surface of a receptacle, and after a short time transfer it to a glass slide, and examine under a high power : there will then be seen numerous motile **antherozoids** of elongated, slightly curved form, and they are kept in active motion by two **cilia** attached to the anterior end. In order to make them clearly visible they should be killed and stained by adding a small quantity of iodine solution, or of osmic acid.

XVI. Add a drop of water containing living antherozoids to a fresh preparation of a female receptacle containing mature archegonia : note the directive influence of the archegonia in attracting the antherozoids to the neck, which they enter, and are lost to sight in the mucilage which fills it.

XVII. Cut median longitudinal sections of a female receptacle bearing almost mature sporogonia: mount as before, and note under a low power that the parts of the receptacle remain as before: but observe especially—

1. The **perigynium,** a loose sac-like coat, which arises from the receptacle after fertilisation, and envelops the archegonium during its further growth.

2. The **wall** of the archegonium, now consisting of two layers of cells, and still bearing at its apex the **neck,** which shows signs of withering.

3. The **sporogonium,** an almost spherical body inclosed by the enlarged venter of the archegonium: the following parts of it are to be recognised—

a. The massive conical foot or **seta** at the end remote from the neck, *i.e.* directed towards the base of the archegonium.

b. The **wall** of the remaining portion of the sporogonium (**capsule**), consisting of a single layer of cells.

c. The hemispherical **sporogenic mass,** with no central columella: in it may be recognised (i.) the **elaters,** long spindle-shaped cells, arranged in a fan-like manner as seen in section; and (ii.) the **spores.**

Mount a small portion of the yellow flocculent mass which escapes on the rupture of a mature sporogonium, and breathe gently on it, observing it the while under a low power: note the hygroscopic movements of the spirally thickened elaters, and the consequent separation and scattering of the spores.

Sections should also be cut through female receptacles of various ages, and by a comparison of these the development of the

sporogonium may be traced from the first divisions of the fertilised ovum to the mature condition.

For comparison with *Marchantia* the sporogonia of some species of *Jungermannia* should also be examined : these grow vertically upwards from the green thallus, and consist of a dark spherical **capsule**, and a more or less elongated transparent **stalk**, or **seta**, the base of which is surrounded by the **calyptra**. When mature, the wall of the capsule splits into four, and the flocculent mass of spores and elaters is set free.

Cut median longitudinal sections of a sporogonium before the stalk is fully elongated, and observe—

1. The parenchymatous structure of the stalk, which is inserted by an enlarged foot in the tissue of the oophore.

2. The capsule, consisting of a **wall** composed of two layers of cells, which incloses the mass of **spores** and **elaters**.

It will be well also to compare the sporogonium of *Anthoceros*, noting the foliaceous irregularly-branched thallus, the upright, elongated, and cylindrical sporogonia, which are inserted on the upper surface of the thallus, and are surrounded at the base by a sheath. The oldest sporogonia may have begun to split into two equal halves from the apex downwards.

Cut median longitudinal sections of a sporogonium of medium age, and observe the enlarged **foot** with hair-like outgrowths penetrating the tissue of the oophore ; the **zone of basifugal intercalary growth** immediately above this ; the **capsule** above will be seen to consist of a **wall** four or five layers of cells in thickness, and having stomata ; a **sporogenic layer**, in which the division of the cells into four may be easily recognised ; and a thin central **columella**. Transverse sections will show that the wall is thinner at two points than elsewhere, and it is here that the rupture takes place when mature.

THALLOPHYTA.

FLORIDEÆ.

POLYSIPHONIA FASTIGIATA, *Grev.*

I. This seaweed is found on all our coasts, growing in dense reddish-brown tufts, which are fixed firmly on to the thallus of *Ascophyllum* (*Ozothallia*) *nodosum*, Le Jolis. It grows to a length of about two inches, and the thin cylindrical thallus is frequently branched in an apparently dichotomous manner: on some of the plants taken in autumn there may be recognised with the naked eye, or with a simple lens, roundish bodies borne laterally (**cystocarps**); on others irregular yellowish tassels at the ends of the branches, these are the **antheridia,** and they are best seen on specimens taken in early summer; on others again dark irregularly disposed swellings may be recognised in the substance of the thallus, these are the organs of vegetative reproduction (**tetraspores**).

II. The material to be used for microscopic investigation should be either quite fresh, and be kept, and mounted in salt water, or better in weak glycerine

or it should be treated while quite fresh with a solution of picric acid in salt water (cf. Part. I. page 2), and after washing with sea-water, be hardened in successively stronger alcohol from 40 to 90 per cent.

From material thus treated, select a thallus which does not apparently bear any of the reproductive organs above mentioned; mount a piece of it, including the tips of some of the branches, in 50 per cent. glycerine and water, and observe under a low power—

1. The cylindrical form of the thallus, and the slight inequality of the apparently dichotomous branching.

2. The general structure of the mature parts of the thallus, consisting of—

a. A series of large **central cells,** with dark reddish-brown contents: these are surrounded by—

b. A single layer of elongated **pericentral cells,** which are arranged with considerable regularity in rings, each ring corresponding to, and surrounding one of the central cells: the whole thallus is thus built up of successive **tiers of cells.**

3. Observe also the apices of the branches, which taper off to fine points, each terminating in a single dome-shaped **apical cell.**

Select a good specimen of an apex, and examine it in detail under a high power: observe—

i. The conical ending of the branch, covered by a thick mucilaginous wall, which extends backwards over the more mature parts, and is covered externally by a definite and continuous **cuticle.**

ii. The single dome-shaped **apical cell,** with highly refractive protoplasmic contents, and more or less obvious nucleus.

iii. The successive segments which have been cut off from its base by parallel transverse walls.

iv. The subdivision of the segments by longitudinal walls, so that each segment ultimately forms one of those tiers of cells (consisting of one central and numerous pericentral cells) of which the whole thallus is built up.

Compare a number of apices in order to ascertain the mode of branching: it will be seen that in some apices the apical cell will have divided by an **oblique** instead of a transverse wall: both of the two cells thus formed gradually assume the properties of apical cells, dividing in the usual way, and growing out right and left as almost equal branches; but the inequality of origin is still to be traced even in old branchings: it is thus obvious that this is not a typical dichotomous branching, but **a form of monopodial branching** closely allied to it.

A perfectly typical example of dichotomy is to be found in *Dictyota dichotoma*, and it may be easily observed on mounting a piece of the thallus in weak glycerine, and examining under a medium power.

In mature parts of the thallus, as also near the young apex, note carefully under a high power the fine protoplasmic strands, which extend through the swollen cell-wall, connecting the protoplasmic body of the various cells of the thallus one with another: for further study of these recourse must be had to sections of the thallus.

III. Embed, and cut transverse sections of the thallus of *Polysiphonia*, selecting such a part of it as is not too old, *i.e.* about half-way between the apex and the base:

mount in glycerine, and examine under a high power: it may then be seen in those sections which pass through the middle of one of the tiers of cells above noted that—

1. The section is circular, since the thallus is cylindrical.

2. That it is limited externally by a clearly marked **cuticle,** and it will be remembered that as *Ascophyllum* on which it grows is found about half-tide mark, the plant is exposed to the air for several hours in each tide.

3. The series of **pericentral cells,** of variable number, each surrounded by a massive, stratified cell-wall, and having a dense, highly refractive, protoplasmic body.

4. The single large **central cell,** having similar characters of wall and protoplasm to the pericentral cells.

5. Note especially the **protoplasmic strands,** which run from the central cell to the several pericentral cells, traversing the cell-wall.

The recognition of these connecting strands of protoplasm between the cells of the thallus of the Florideæ dates back to a period before "protoplasmic continuity" had acquired the special interest which it now possesses; nevertheless, there is still some difference of opinion as to the details of their structure: some hold that, at least in some cases, the strands are continuous, without any break, from one cell to another; that this is not the case universally is obvious, as **pit-membranes** may be seen (and clearly in the majority of cases in *P. fastigiata*) traversing the strands transversely. It has further been asserted that, where this is the case, the pit-membrane has the character of a **sieve**; but still Schmitz holds that there is no exchange of protoplasm

as such, since the pit-membrane is covered on either side by thick plates of a highly-refractive substance ("stoppers" of Wright), which are themselves, however, connected by fine strands passing through the pit-membrane.

Valuable information on various points is to be gained by treating fresh specimens of thalli, which do not bear reproductive organs, with slightly dilute sulphuric acid for about an hour: material thus prepared is to be mounted in water or weak glycerine, and by a slight pressure with a needle on the cover-slip the protoplasmic bodies of the cells may be disengaged from the greatly swollen cell-walls: they will often be seen to hang together in groups, and the fine protoplasmic strands (with a more or less obvious sieve arrangement) will then be seen extending between them: a subsequent staining with Hoffmann's blue will make this more apparent. Special attenton should be given to the subdivision of the successive segments cut off from the apical cell, since the pericentral cells are derived from them in a peculiar manner. It may be seen that they are formed, not simultaneously, but **successively.** The first is cut off in form of a wedge, and the rest of the pericentral cells appear alternately on either side of the first, until the whole periphery of the segment is occupied by a series of pericentral cells surrounding the central one from which they are derived.

A careful observation of young apices externally, under a high power, will be found to bear out this observation.

IV. Longitudinal sections should also be cut so as to complete the study of the tissues: in median longitudinal sections the series of central cells will be obvious under a low power, the dark protoplasmic body being surrounded by a swollen and beautifully stratified cell-wall. Externally will be seen the pericentral cells of oblong form, their free walls being covered by the continuous cuticle. Pay special attention, under a high power, to the protoplasmic strands, which connect the cells of the central series one with another, and also with the pericentral cells. The former are thick strands,

with large and obvious "stoppers" on either side of the pit-membrane; the latter are much thinner, and the presence of the pit-membrane, and "stoppers" is consequently less easy to observe, if indeed they be constantly present at all.

V. Turning now to the insertion on the frond of *Ascophyllum*, detach the bases of one or two of the thalli of *P. fastigiata* from the substratum by means of forceps, noting the firmness of their attachment: mount, and examine under a low power. It will then be seen that towards the base of the thallus single superficial cells are of elongated, cylindrical form, and have thick walls.

VI. Cut transverse sections of the frond of *Ascophyllum*, so as to pass through the insertion of one of the tufts of *Polysiphonia:* mount as before, and observe under a low power that those elongated, thick-walled cells above described penetrate deeply into the thallus of the host, and thus obtain a firm hold upon it, while their own strongly thickened walls will explain further the strength of attachment.

For comparison with the above type, observations should also be made on other forms among the Florideæ, and the following are suggested as good illustrative examples:—

A. *Batrachospermum moniliforme*, Roth., a purplish-brown, slimy-feeling Alga, which grows on stones in fresh-water streams. Observe it fresh, mounted in water, or preserved in weak glycerine, and note under a medium power the **central series** of cells, which terminates in a dome-shaped apical cell: in the mature parts, the cells of the central series are elongated, as internodes; while at the nodes are inserted brushes of branched multicellular filaments, often terminating in long fine bristles: in the older parts, some of these filaments are closely applied to the internodal cells, thus forming a sort of cortex.

B. *Ptilota plumosa*, Ag., a small, dark-purple seaweed, found growing on vertical or overhanging rocks: mount in glycerine, or, if fresh, in sea-water. Observe a well-marked central series of cells, which are individually short, and there is a single apical cell as before: branches are inserted, in one plane, on either side of the central series: in the older portions a cortex surrounds the central cells, and it may be traced as originating partly by outgrowth and division of the central series, partly from the bases of the branches.

C. *Chondrus crispus*, Stack (Irish Moss or Carragheen) should also be observed as an example of a more massive thallus; it is very variable in outline, according to the conditions under which it grows: the branching is dichotomous, and the thallus more or less broadly strap-shaped, being attached by a disk-like organ to the substratum.

Cut transverse sections of the thallus, and mount in pure glycerine: examine under a high power, and note—

1. The superficial cuticle.

2. The outer cortex, composed of linear series of small cells.

3. The inner cortex, consisting of larger cells, with swollen cell-walls, which are traversed by protoplasmic threads: these are, however, usually (if not always) intercepted by a highly refractive pit-membrane, the small size of which makes it difficult to trace any sieve-like structure.

4. A central mass of hyphal tissue.

Thus there is no central linear series as in the plants previously described: the whole structure approaches rather to that of *Fucus* (see below).

D. *Corallina officinalis*, L., should also be examined as a type of the lime-incrusted Corallines: it is common between the tide-marks on all our coasts. Note the mode of branching of the thallus and its calcareous consistency. Sections should be cut from the tissues, either after dissolving the lime with acetic acid, or, better, by grinding as in the preparation of geological sections: in the latter case observe—

1. The successive pear-shaped joints of lime-incrusted tissue: these can be recognised externally with the naked eye.

2. The intervening transparent zones of tissue with pliant mucilaginous walls.

The whole is made up of cells of nearly equal size, arranged in linear series, which correspond to one another in such a way that the cells appear in fan-like zones. Here again there is no single central series larger than the rest.

E. Observations may also be made with advantage on specimens of *Lemanea*, a genus of dark olive-green, fresh-water Algæ, included in the Florideæ : these plants grow attached to rocks in mountain streams. Note especially—

1. The simple filamentous, and branched proembryo, similar in many respects to that of *Chara :* by means of this the plant is attached to the rocks.

2. The more complex "reproductive thallus" which is produced as a lateral branch upon it : in the character of the apex, and in its mature structure, it is not unlike that of some species of *Polysiphonia :* it has clearly marked nodes.

Transverse and longitudinal sections should be cut, from which it will be seen that the "reproductive thallus" consists of a central series of elongated cells, which traverses a cavity filled with mucilage, and is connected by lateral struts, at points between the nodes, with the cortical tissue : the latter consists of relatively large internal cells, and relatively small superficial ones.

VII. Having distinguished with a lens, or under a low power, a specimen bearing tetraspores, mount a portion of it as before directed (taking care that young branches as well as mature ones are represented), and examine under a medium or high power. Note—

1. That the regularity of the tissues of the thallus is disturbed at certain points by dark spherical bodies lying embedded in the tissue below the pericentral cells : these are the **tetraspores.**

2. That they decrease in size as the apex of the branchlet is approached.

3. That they have no clearly definite arrangement.

4. That each undergoes a division into four, hence the term "tetraspore."

5. That they escape by rupture of the layer of pericentral cells: note in older parts the vacant cavities whence tetraspores have escaped.

6. That the mature tetraspores are naked, spherical, and motionless protoplasmic bodies.

By a careful comparison of tetraspores in early stages of development it may be ascertained that they originate from cells of the central series, as shortly-stalked bodies, of which the head enlarges and divides into four, while the pedicel remains small.

Though the term "tetraspore" is strictly applicable in this case, as in most of the Florideæ, still in *Callithamnion* the mother-cells may undergo a variable number of divisions, or may even remain undivided.

In many forms, with simple structure of the thallus, the tetraspores may be produced externally, as in *Callithamnion*, *Ptilota*, &c.

VIII. As above noted, the male plants may be recognised by the presence of yellowish tassels of antheridia, which are to be found mature from the beginning of April onwards: late in the season the ends of their branches become ragged and irregular. Mount as before a small piece of a male plant taken in April, and observe under a medium power—

1. The normal structure of the main thallus.

2. The club-shaped **antheridial branches,** often associated together in groups: each consists of—

a. A unicellular **pedicel,** by which it is attached to the thallus.

b. A central linear series of cells, which is almost entirely hidden by—

c. Numerous, closely aggregated, and small **antheridial cells.**

Mount specimens of antheridial branches from fresh living material in sea-water, and having found an antheridial branch exactly at the period of maturity observe the partial disorganisation of the walls of the antheridial cells, and consequent liberation of their protoplasmic contents, without subdivision, as round non-motile **spermatia**.

A comparison of numerous antheridial branches in various stages of development will demonstrate that they originate from single cells of the thallus, close to the apex of the branch: these cells divide repeatedly by transverse walls, so as to form a linear series, around which, in place of the normal pericentral cells, antheridial cells are cut off by repeated longitudinal or oblique divisions from all the cells of the linear series, except the basal one, which remains as the pedicel.

IX. Having recognised a female plant by observations with a lens on specimens taken in late summer or autumn, mount a portion of it in glycerine, and, examining it under a low power, observe—

1. The normal structure of the thallus, which bears—

2. **Cystocarps** of ovate form: these consist of closely aggregated, small-celled tissue: they occupy the same position as the lateral branches in a vegetative thallus.

3. Compare numerous specimens, and note cystocarps in various stages of development.

By careful observation on material taken in early summer the various steps in the development may be traced, such as the origin of the cystocarp as a multicellular body seated at a point immediately below the apex of a short lateral branch of the thallus, and recognised as a darker group of cells: the mode of

segmentation of the young cystocarp, and the formation of the **trichogyne** as an elongated thread-like cell, which overtops the apex of the branch on which it is borne; its fertilisation by a spermatium, and the further growth of the cystocarp.

As examples of simpler structure of the fruit may be suggested *Batrachospermum*, and *Polyides* or *Dudresnaya*, the latter genera being specially remarkable for the indirect character of the process of fertilisation.

The case of *Lemanea* is worthy of note: the carpogonia are formed internally, but with a projecting trichogyne. In summer the carpospores are to be found borne on branching filaments which occupy the large mucilage cavities in the thallus.

In the forms with more complicated structure, the cystocarp is often embedded in the tissue of the thallus: sections should be cut to illustrate this in *Chondrus crispus*.

X. Embed mature cystocarps of *P. fastigiata* in paraffin, and cut from them median longitudinal sections: mount in glycerine, and observe—

1. The short thick stalk of the cystocarp.

2. Its wall, consisting of small, closely aggregated cells, and with an opening or **ostiole** at the apex.

3. The central cavity, surrounded by the wall, and filled more or less completely, according to age, with elongated, club-shaped cells, having dark protoplasm, and swollen walls: these are the **carpospores.**

The following points are further to be observed in longitudinal sections, viz.—

1. The formation of the carpospores by budding from the base of the cavity of the cystocarp.

2. The contents of the spores in various stages of escape from their cell-walls, and of passage through the ostiole, so as to escape freely, as rounded and naked protoplasmic bodies, out from the cystocarp.

The artificial germination of the spores is a

matter of difficulty, but a rough idea of the germination of the spores of red seaweeds may be gained by observing the numerous young plants, of various genera and species, which are to be found attached to the outer surface of almost any one of the larger seaweeds.

PHÆOPHYCEÆ.

FUCUS SERRATUS, *L.* (Wrack).

OBSERVATIONS WITH THE NAKED EYE.

I. Of the various species of *Fucus* which are to be found on our shores, the best adapted for laboratory work is *Fucus serratus:* it is to be found near or below mid-tide level, and may be distinguished from other species by its dark olive colour, the flattened form and serrate margin of the branches of the thallus, the absence of swollen "bladders," and the presence of numerous dot-like conceptacles, crowded together on the ends of branches which show **no special swelling.**

Having recognised the species by these characters, examine a well-developed plant with the naked eye, and note that the **thallus** as a whole shows no differentiation of stem, leaf, and root, as in vascular plants; it consists of the following parts—

1. The flattened **disk,** of irregular outline, by means of which the plant is firmly attached to the substratum: the attachment is at times so firm that the stalk itself will break before the attachment gives way.

2. The **stalk,** which in old plants is of compressed cylindrical form, but in young plants it may be clearly

seen that it is originally a broad flattened expansion, with a more or less thickened midrib: a comparison of plants of successive ages will demonstrate that the compressed cylindrical stalk results from the thickening of the midrib, and decomposition of the lateral wings.

3. If this stalk be traced upwards it will be seen to branch repeatedly, while on tracing the branches upwards they gradually assume the flattened form with serrate margin, thus confirming the conclusions which may be drawn from a comparison of younger plants, viz. that the whole thallus, when young, was of a flattened form, and that after growth and repeated branching the lower portions assumed the flattened cylindrical character, by thickening of the midrib and loss of the lateral wings.

The following observations are also to be made—

A. Examine the **apices** of young, actively-growing branches: those branches which bear conceptacles must be carefully avoided, as they do not show such characteristic appearances: the extreme apex is emarginate, or depressed, the base of the depression being somewhat flattened, and marked by a slight groove running in the plane of the thallus: it will be seen subsequently that the **initial cells** lie at the bottom of this groove.

B. Compare a number of apices: in some only a single emargination will be seen, in others two, similar to one another, each having the groove at the base, while others again will show an intermediate appearance: from this it may be concluded that the single apical point divides into two of equal strength,

each of which may develop into a branch of the thallus similar to the original: thus the **branching is a dichotomy.**

C. On comparison of a number of branches it may be seen that the development of the two branches of a dichotomy is not equal, one being usually stronger than the other: this leads to a **sympodial development of the dichotomous branch-system.**

D. Observe the less regular outline of the ends of those branches which bear **conceptacles,** the latter being seen in large numbers on those branches: note with a lens the round orifice or **ostiole** in the centre of each conceptacle. When mature, two kinds of conceptacle may be recognised in this species; they are borne on different plants: and this species may accordingly be recognised as dicœcious.

a. Conceptacles the contents of which are of a dark olive-green: these contain the **oogonia,** and are the **female conceptacles.**

b. Others with yellow or orange contents: these are the **male conceptacles,** and contain the **antheridia.**

Of other common species of *Fucus*, it is to be noted that in *F. vesiculosus* there are numerous air-bladders in the tissue of the thallus, that the conceptacle-bearing branches are swollen, and that the distribution of the sexual organs is as in *F. serratus*, the plant being dicœcious: in *F. platycarpus*, on the other hand, male and female organs are found on the same plant and in the same conceptacle, while the fertile branches are, as in *F. vesiculosus*, considerably swollen.

E. If those flattened parts of the thallus be examined which do not bear sexual conceptacles, there will be found, scattered here and there, organs of somewhat

similar structure, which contain only barren hairs, and they may be termed **sterile** or **neutral conceptacles** (*fasergrübchen*).

F. Note the dark olive-brown colour of the whole thallus when fresh. Place a piece of the fresh thallus in methylated spirit: in a short time it will assume the bright green colour characteristic of chlorophyll: this is due to the fact that the brown colouring-matter (phycophæin, or phycoxanthin) characteristic of the Phæophyceæ, masks the chlorophyll in the natural state; but, being more readily soluble than chlorophyll, it is first removed, and the chlorophyll is then apparent.

MICROSCOPIC EXAMINATION.

II. As it is almost impossible to make satisfactory preparations of the tissues of *Fucus* from fresh material, it will be found a great advantage to fix and harden them, by some such method as that described in small type in Part I. page 2: by this method the material is fixed by treatment with a solution of picric acid in sea-water, and after washing, to remove excess of picric acid, it is hardened in successive strengths of alcohol. The length of time of immersion in the picric acid should be proportional to the bulk of the tissue, so that the reagent may penetrate throughout the tissue.

An alternative method, which gives good results, is to treat the specimens first with 1 per cent. chromic acid, wash with water, and harden in successive strengths of methylated spirit.

From material thus prepared select a young flattened branch of the thallus in which the midrib is but slightly

marked: cut transverse sections from it, mount in pure glycerine, and observe under a low power—

1. The elongated elliptical outline of the section.
2. The more or less enlarged midrib.
3. The grouping of the tissues exposed: recognise especially—

a. A compact marginal band of tissue of a yellowish-brown colour, the **cortical band**: this graduates off into—

b. The less compact central mass of the **medulla,** consisting of a web of interlacing filaments.

Put on a high power, and examine the tissues in detail. Starting from the periphery, observe that the cortical band consists of—

1. A superficial or **limiting layer** of cells, regularly arranged, and elongated radially; the cells are not of uniform depth, and examination will show that they divide by periclinal, as well as by anticlinal walls; in fact they constitute an active, continuously meristematic layer, and accordingly the term "epidermis" cannot be applied to it in the strict sense. With the exception of the outer wall, which is thick and cuticularised, the walls of these cells are thin, and the protoplasm plentiful, with a nucleus.

Treat a section with strong sulphuric acid: all the cell-walls swell, and lose their definite outline, with the exception of the outer layer of the superficial wall, which remains sharply marked. Treat a fine section with Schulze's solution: the cuticle will stain yellow, while the walls of the internal tissues stain a pale blue.

2. Immediately below the limiting layer, and separated

from it by an irregular line, is a parenchymatous tissue, consisting of cells which appear nearly square in the transverse section; each cell has plentiful protoplasm, a nucleus, and several chlorophyll corpuscles: the walls are more or less thickened, swollen, and stratified: here and there are to be seen **pits** closed by a thin, highly-refractive pit-membrane. These two tissues (1) and (2) together constitute the **cortical band** above recognised under a low power.

Here, as in other cases of pitted tissues, the question of perforation of the pit-membrane has been raised: though the actual proof is in this case difficult, it is asserted by various authors that the continuity of protoplasm through the pit-membrane has been actually observed.

3. The above tissue graduates off without definite limit into the **medulla,** of which the chief characteristic is the excessive bulk of the swollen cell-walls: an idea of the manner in which this comes about may be formed by following the gradual transition from cortex to medulla, when it will be apparent that the parts of the wall adjoining the middle lamella (and sometimes the middle lamella itself) swell greatly to form a gelatinous matrix, in which the cells appear to be embedded, each being surrounded by a definite, firm, cellulose wall. Here and there "trabecular" filaments will be found running in the plane of section. If sections be stained with Schulze's solution the firmer cell-wall stains pale blue, but the swollen matrix does not stain.

Occasionally a section may be found which has passed through one or more of the sterile or neutral

conceptacles (*fasergrübchen*) ; these will then appear (if cut through the ostiole) as an irregularity of the cortical band. If the conceptacle was young, it would be found to be a still closed cavity of considerable size, filled with mucilage, and with hairs which originate from single cells of the tissue lining the cavity ; in sections of older parts the ostiole would be found widely open, the hairs much longer, and protruding out of the conceptacle : these hairs may be observed even with the naked eye on the older parts of the thallus.

III. Cut longitudinal sections of a young part of a thallus (*i.e.* close to the apex of a branch), mount in pure glycerine, and examine under a high power : recognise, as in the transverse sections, the **cortical band,** consisting of—

1. The **limiting layer,** which presents similar characters to those seen in transverse sections.

2. The **inner parenchyma :** note that the cells of the inner part of this tissue are arranged in longitudinal rows, having relatively thick, occasionally pitted, lateral walls, and thin transverse septa : this tissue merges gradually into—

3. The **medulla,** which consists of **"trabecular" tissue :** here longitudinal rows of cells constitute filaments resembling the hyphæ of Fungi, with numerous thin transverse septa, to which the protoplasmic contents closely adhere : as already seen in transverse section, the longitudinal walls consist of a firm, highly refractive cellulose coat embedded in a mucilaginous matrix. Note the frequent lateral communications of the filaments, and compare them with the pits in the cortical tissue, to which they undoubtedly correspond.

IV. In order to study the process of thickening of the midrib, cut transverse sections successively of older (*i.e.* lower) parts of the stalk: treat as before, and compare them. It will then be recognised that, as the thallus grows older, the cells of the limiting layer cease to divide by periclinal, and later also by anticlinal walls: it becomes a quiescent tissue, and is ultimately thrown off; the inner cortical tissue however remains active, the cells increase in size, dividing periclinally, and form a massive band, easily recognised by the naked eye. The medulla also increases greatly in bulk, many new hyphal filaments being formed, while they differentiate into two series: (*a*) smaller ones, with sparing protoplasm; (*b*) others of larger size, with a granular protoplasmic lining.

Longitudinal sections made at different points down to the thickened stalk itself, will lend solidity to these observations: in such sections note, especially in the oldest parts of the stem, the different types of hyphæ; also the origin of fresh hyphal filaments from cells of the cortical band, as tubes which push their way through the mucilaginous cell-walls of the earlier-formed tissue. Look for endings of some of the filaments: note also the circular pits in their lateral walls.

V. Sections should be cut through the organ of attachment. Take plants grown on wooden piles, or on limestone rock: in the latter case the lime may be dissolved by acetic acid, and the tissue then hardened in alcohol. Cut vertical sections, and mount as before: note under a low power the irregularity of the surface of attachment, which closely follows that of the substratum, hence the firmness of its hold. Foreign bodies

may often be seen embedded in this part of the thallus, and this finds its explanation in the fact that the tissue here consists of hyphæ similar to those of the medulla, and each appears to grow in an independent manner. Examine the section under a high power, and it will be seen that the mass of tissue resolves itself at the surface of attachment into a number of separate filaments, each of which applies itself separately to the surface of the substratum. Compare drawings or preparations of young plants (see below, page 62).

VI. Cut thin longitudinal sections through the apex in a plane perpendicular to that of the flattened thallus: treat those sections which are **median** with glacial acetic acid, and mount in a mixture in equal parts of pure glycerine and glacial acetic acid: examine under a medium power, and observe—

1. The outline of the section, showing a depression of the apex corresponding to the groove already recognised with the naked eye.

2. That from their arrangement it may be concluded that the various tissues of the thallus are derived from an initial point at the base of the depression.

3. Trace the cuticle as continuous from the free outer surface into the slightly enlarged cavity of the depression, which is itself filled with a mucilaginous secretion lying between the cuticle and the cellulose walls of the adjacent cells.

4. Examining the tissue at the base of the depression, if the section be median, a single large cell having the form of a truncated pyramid may be recognised in a central position: **this is one of the initial cells.**

Put on a high power, and observe the **form** of the initial cell, and its relation to the adjoining tissues: it will then be noted that segments are cut off alternately from either side, and then one from the base. The lateral segments undergo further divisions according to the type of the limiting layer; the basal segments divide up, and ultimately pass over into medullary tissue.

If sections through the apex be stained with hæmatoxylin, it may be observed that each cell contains a single **nucleus**: this observation may also be extended to the tissues of the older parts of the thallus.

VII. Cut successive transverse sections of the apex, so as to pass immediately below the base of the depression: one of these sections will include **the initial group,** which will then appear to be composed of some four or five cells, of oblong form, placed in a row, side by side.

Examining these under a high power it may be seen that segments are cut off (1) from either side of their oblong outline, (2) from the sides of those of the initial cells which are at either end of the row, and (3) that any of the initial cells may divide by a median wall into two similar cells: by the latter process the number of initial cells may be increased, and this is usually the case before dichotomy, which is brought about by the cells at the middle of the row ceasing to act as initial cells, and passing over into the condition of permanent tissue, while those at either end of the row retain the character of initial cells, and are to be recognised as the two independent initial groups of the new branches: thus the branching is a dichotomy. The attempt should be made to find sections illustrating this process of branching.

This type of apex is by no means constant for the Fucoids; thus in *Himanthalia* there has been found in each branch of the thallus only a single apical cell, which appears triangular in transverse section, but in longitudinal section it shows two convex sides: the form of the cell is thus, roughly speaking, that of a Brazil nut.

VIII. Cut transverse sections through the fertile branches of the thallus, so as to traverse the mature conceptacles: mount in glycerine, and examine under a low power. Take first sections of the male thallus, and having found a point where a male conceptacle has been cut in median section (*i.e.* so as to traverse the ostiole), note—

1. The spherical, or flask-shaped **cavity.**

2. The **ostiole,** by which the cavity communicates with the exterior.

3. The **hairs,** which almost fill the cavity, and may even protrude through the ostiole.

4. The **antheridia,** which are single oval cells, borne often in large numbers, on these hairs.

It may be further noted that the cavity is lined by a small-celled tissue, from which these hairs arise, and that this graduates imperceptibly into the other tissues of the thallus, which are similar to those of the purely vegetative parts.

IX. Tease out with needles the contents of a male conceptacle in glycerine, mount, and observe under a high power: note—

1. The thin, colourless, branched hairs, which bear the oval cells (**antheridia**) with their yellowish granular contents.

2. The mode of branching of the hairs which bear the antheridia.

3. Long hairs, branching less frequently, or not at all; these do not bear antheridia.

X. With the above, compare sections cut through female conceptacles, mounting as before: in its form, and also in its relation to the tissues surrounding

it the female conceptacle is similar to the male; the difference is in the contents, which may be seen to consist of—

1. Barren hairs, which are usually unbranched.

2. **Oogonia,** bodies of relatively large size, and oval form, with a thick transparent wall, and dark granular protoplasm: each of these is seated on a unicellular pedicel, and may be regarded as a metamorphosed hair.

Observe in the largest of the oogonia that the protoplasmic body may be seen to have undergone division into eight parts, the surfaces of separation being visible as transparent lines.

In order to follow the development of the conceptacle it is necessary to cut sections from the apical region of a branch in which the formation of conceptacles has recently begun: median longitudinal sections will be found to be the best, since they will often show a series of successively younger conceptacles leading up to the organic apex. From a comparison of these it will appear that the conceptacle is not a mere involution of the surface, but that one (or in some cases more than one) cell of the limiting layer shrivels, and is thrown off: that the walls around it undergo mucilaginous swelling, which is probably connected with the formation of the flask-shaped cavity: that the conceptacle is closed until after the hairs begin to be formed from the tissue lining it, when ultimately the ostiole opens to allow the escape of the sexual cells.

XI. Observations on the extrusion of the antherozoids and oospheres, and on the process of fertilisation in *Fucus* must be made with fresh material, and will be most successfully carried out on the coast, the best season for it being winter or spring. Those who have not opportunity for this may succeed in making the observations on fresh material sent from the coast, using

a solution of Tidman's sea salt of proper strength, in place of fresh sea-water.

If specimens of *Fucus serratus* be kept exposed to the air for some hours (the period of one tide will suffice), an exudation may be observed from the ostioles of some of the conceptacles: on male plants it will be of an orange colour, on female plants of a dark olive-green.

Taking first the male, mount a small quantity of the orange exudation in a drop of fresh sea-water, and examine it under a high power: it will be found to consist of numerous **antheridial cells,** separated from the hairs which bore them: they will be seen to be bursting, and setting free their contents, and the following stages of the process are to be noted—

1. The antheridial cell is completely closed, the contents are already divided into numerous elongated bodies (said to be sixty-four in number), each having one or sometimes two brightly orange-coloured globules (**chromatophores**): these are the **antherozoids,** and they may be seen to be in motion before the antheridium bursts.

2. The wall of the antheridium consists of two layers, the outer more firm layer (**extine**) and the inner mucilaginous layer (**intine**): observe the extine to burst at one end, usually at the apex, and the contents inclosed in the intine escape from it.

3. The intine gradually swells, loses its contour, and the antherozoids separate, as actively motile bodies of elongated pear-like form. Observe their movements.

To a drop of water containing motile antherozoids add a little iodine solution, put on a cover-slip, and

examine under a high power: the two **cilia** may be observed attached laterally.

Mount in a drop of fresh sea-water some of the darker-coloured exudation from the female conceptacles, and examine under a high power: observe the numerous **oogonia,** with the pedicel cell often attached: note the thick limiting wall, consisting obviously of two layers, an outer (**extine**) more highly refractive, the inner (**intine**) having the characteristic optical appearance of a mucilaginous wall: a shallow pit is to be seen on the wall adjoining the pedicel. The contents will be seen in most cases or in all to be divided, as above described, into eight cells—the **oospheres.** Some of the oogonia will be seen to burst on exposure to the water: watch the process, and note the following stages—

1. A slight convexity appears, usually near the apex, the extine having there ruptured, and the intine beginning to protrude.

2. The rupture extends, and the extine gradually shrivels back so as to leave the intine fully exposed, though it usually remains still attached to the extine at the base.

3. The intine swells, and ultimately loses its contour at the apex, and the oospheres, which had meanwhile separated and rounded off, escape into the water as eight naked, non-motile spheres of dark granular protoplasm: in each may be recognised a central clearer area—**the nucleus.**

Into a drop of sea-water in which are free and mature oospheres, introduce a small number of mature antherozoids, and watch their movements: they may

be seen to approach the oospheres, to apply themselves closely to their surface, along which they creep: if present in considerable numbers, they give to the oospheres an irregular rotating movement.

Actual coalescence of the antherozoid with the oosphere is difficult to observe, and it is even doubtful whether it has ever been seen: the appearance of a second nucleus in the oosphere has, however, been described, and its coalescence with the nucleus of the oosphere: it is probable that if an antherozoid, as such, enters the oosphere, its entry is very rapid.

XII. The most satisfactory way of tracing the development subsequently to fertilisation is by artificial cultivation: this has been done by Rostafinsky, so as to prove that the fertilised ovum develops directly into the *Fucus* plant; but the cultures, being difficult to carry out, are not fitted for class demonstration. It is thought that the following simple observations will suffice.

On stones, in districts where *Fucus* abounds, there may be found in early summer olive-brown velvety patches: on examining these with a lens, small club-shaped bodies may be distinguished, attached by their narrower end to the substratum, and with their broader, free end crowned by a tuft of hairs: these are young plants of *Fucus*, or of one of the allied genera.

Having collected such material, and treated it as above directed, tease it out with needles, in glycerine, and examine the plants thus separated under a low power: the following points are the most worthy of note—

1. The nearly spherical form of the very young

plants, which consist of but one, or of relatively few cells, and are limited externally by a definite cell-wall. the formation of this wall is the first obvious change after fertilisation.

2. The elongated club-shape of the older plants.

3. Their terminal depression, from which numerous hairs protrude.

4. The mode of attachment by means of hypha-like threads of independent growth, similar to those seen in the older plants: some of these threads may be seen quite young, and not yet attached to the substratum.

From such plants as a starting-point, intermediate forms will lead on to the mature *Fucus* plant.

Laminaria digitata, *Lamour.*

The genus *Laminaria* is so prominent among the Phæophyceæ that it calls for a short notice: the various native species of the genus grow near to or below the low-tide mark, and *L. digitata* is perhaps the commonest of them all.

Note with the naked eye in fully-grown specimens of this plant—

1. The **organ of attachment** at the base, which produces many branches: these approach the substratum, and attach themselves closely to it.

2. The elongated **stipe,** of more or less flattened cylindrical form, with a brown exterior and cartilaginous consistence.

3. The expanded **frond,** which is also cartilaginous, and is irregularly cleft into segments.

4. On some specimens, especially in winter, may be found irregularly thickened, slightly brownish patches, on which the reproductive organs are produced.

Cut through the stipe transversely, and, smoothing the surface, examine it with the naked eye: if the plant be an old one, there will be seen—

1. A brown peripheral band of outer cortical tissue.

2. A massive zone which constitutes the chief bulk of the stipe: in the centre of it may be recognised—

3. A central medullary patch of narrow-oval form, which indicates the originally flattened character of the stipe itself.

From material previously hardened in alcohol, cut transverse sections of the stipe, so as to include all the tissues to the centre: mount in glycerine, and examine under a low power: note—

1. The superficial tissues of the **external cortex**, which may be more or less brown according to age, both in the walls and the cell-contents. In old specimens there will be found large **mucilage-cavities** at the inner limit of this tissue, which are apparently of schizogenetic origin.

2. A meristematic zone, or **cambium**, in which the cells correspond in appearance, and in their arrangement in radial rows, to those of the cambium of vascular plants: by the activity of this layer the stipe undergoes a **secondary thickening**.

3. Passing inwards, a bulky mass of ground tissue is found consisting of cells arranged with considerable regularity in radial rows, pointing to an origin from the cambium: the cell-contents are for the most part inconspicuous, and the walls of somewhat irregular thickness.

4. Centrally is the oval area or **medullary tissue**, above noted with the naked eye: the tissue here is traversed by numerous **hyphal threads**, similar in nature to those in *Fucus*.

Cut longitudinal sections of the stipe, and, treating as before, observe—

1. That the external cortex, cambium, and ground tissue consist of cells which are of no great length, with rounded or square ends, and walls with a few pits.

2. That the mucilage-cavities do not extend far in a longitudinal direction.

3. That the medullary tissue consists chiefly of hyphal threads closely interwoven, with thick lateral walls and thin transverse septa. Note especially at the periphery of this tissue how the hyphal threads originate as papillæ from the cells of the ground tissue, and then push their way as they grow between the neighbouring cells.

Cut transverse and longitudinal sections of one of the segments of the frond, being careful to avoid the reproductive patches.

Comparing their structure with that of sections of the stipe, observe that the arrangement and character of the tissues is in the main similar: observe further—

1. That there is no definite epidermis (compare *Fucus*).

2. That the character of the cell-walls is like that in *Fucus*, the middle lamella being swollen, and the pit-membranes highly refractive.

3. Where the section has traversed the margin of one of the thong-like segments of the frond indications may be seen of their separation from one another by rupture, since the tissues are irregular at these points, and there is no superficial band as over the rest of the surface of the thallus.

Cut sections through the reproductive patches, and mount in strong glycerine.

Here the superficial cells are more elongated than in the vegetative portion, thus forming a deep layer, in which two different constituents are to be recognised—

a. Narrow club-shaped cells, with swollen walls: these are the **paraphyses.**

b. Shorter cells, with thinner walls, and more oval form: these are the "**sporangia,**" the granular contents of which may be seen to divide into a number of reproductive bodies, the further development of which is unknown.

CONFERVOIDEÆ.

COLEOCHÆTE SCUTATA, *Bréb.*

Various species of *Coleochæte* are to be found growing closely attached to the surface of submerged fresh-water plants: they are green-coloured, and they attain such a size as to be recognisable with the naked eye. The thallus shows considerable variety of structure in different species: thus it may appear as a flattened disk, one layer of cells in thickness, closely attached to the surface of the plant on which it grows (*C. scutata*); or it may consist of a number of filaments branching in one plane only (*C. soluta*); or it may take a hemispherical massive form, being composed of closely packed, branching filaments (*C. pulvinata*). Of these species *C. scutata* is very common, and is best adapted for observation. Having ascertained that this plant is present (*e.g.* on the lower surface of the lamina, or on the petiole of the Water Lily) cut thin tangential sections, removing as little as possible of the tissue of the leaf, but taking care not to injure the Alga: mount in water, and examine under a low power. Note—

1. The flattened discoid thallus, with more or less irregular circular outline: it consists of one layer of

cells, with thin cell-walls, a single nucleus, and a single flattened chlorophyll-body (chromatophore).

2. The arrangement of the cells in radiating and bifurcating series, which are however in close contact laterally one with another, and thus compose a continuous disk.

3. The hairs, which are borne by many of the cells: they are long and narrow, and are sheathed at the base.

According to circumstances, the following reproductive organs may or may not be found :—

1. **Swarm-spores,** each of which is formed from the contents of a single cell of the thallus, by contraction, and escape through a hole in the cell-wall into the water: the swarm-spores are spherical primordial cells; they move for a time by means of a pair of cilia.

2. **Antheridia,** which are formed by division of cells of the disk into four: the contents of these escape as ciliated **antherozoids.** In other species they may be formed from terminal cells of the filaments (*C. pulvinata*).

3. **Oogonia,** flask-shaped cells, with long tubular necks: each oogonium contains one **ovum.**

4. The **fructification,** which results partly from the maturation of the oogonium after fertilisation (**oospore**), partly from its investment by filaments which grow from surrounding cells, forming a sheath one layer of cells in thickness, and of a brown colour when ripe. In this state the winter is passed.

5. In specimens observed in spring the general outline of the thallus will be seen as before, but the cells, excepting those of the fructification, have lost their

contents. Cell-division now takes place in the oospore to form numerous cells, from each of which a swarm-spore may be produced. These may escape by disorganisation of the investments, and, after a period of movement, settle, form a cell-wall, and grow into a new thallus.

The observation of (1), (2), and (3) may be made in early summer; that of (4) in autumn or spring.

ŒDOGONIUM.

Various species of plants belonging to this genus are to be found growing in fresh water: they are green, filamentous, unbranched Algæ, and are attached at the base to the external surface of submerged plants, stones, &c. The apex of the filament is in some species extended into a thin, hair-like process: there is often considerable irregularity in the thickness of the filament, by which character, as well as by the peculiar markings of the cell-walls, these plants may be distinguished.

I. Mount filaments of *Œdogonium* in fresh water, having gently scraped them off from the surface to which they were attached, and examine them under a low power: observe—

1. The long unbranched filament, of uneven thickness, terminated at the apex either by a rounded cone, or by an attenuated process: note also at the base the **irregularly lobed disk of attachment.**

2. The **septa,** dividing the filament into a series of cells, with green-coloured contents.

3. At the upper ends of many of the cells are to be

seen **transverse striæ :** these are indications of past cell-divisions.

Examine the filaments under a high power, and pay special attention to these striæ and other irregularities of the otherwise smooth cell-walls. It will then be seen that the striæ are small, sharp-edged, ring-like projections on the outer surface of the wall : also that a single corresponding stria is to be found more or less distinctly at the base of each cell.

In some cells there may be seen an **annular ingrowth of the cell-wall** immediately below the striæ : note its form and connexion with the cell-wall ; also, when seen in optical section, a central, dark mark : it is here that the ring splits, and by stretching of the ring the well-known process of **intercalation** of a new zone of cell-wall follows. Examine actively-growing filaments, and try to observe various stages of this peculiar process, noting also any indications of cell-division which follows it, the new septum being formed immediately below the thin-walled intercalated zone.

Passing to the examination of the cell-contents, observe—

1. The colourless **protoplasmic basis,** in which are embedded—

2. The **chromatophores,** which appear as elongated and branched rod-like bodies, more or less closely and irregularly connected together : here and there will be seen highly refractive **pyrenoids** attached to the chromatophores : these are clearly to be distinguished by their dusky purple staining on treatment with iodine solution.

3. A single **nucleus** in each cell, which is, however, difficult to recognise in well-nourished cells.

4. A large central **vacuole.**

II. An examination of fresh filaments may result in the observation of the reproductive organs, and numerous specimens should be looked over with the object of finding them. Thus the reproduction by **swarm-spores** may be seen, especially in the morning: without the cell having undergone any change of form the cell-wall ruptures by a transverse split, and the protoplasmic body, having previously contracted, escapes through the slit as a motile pear-shaped, primordial cell, the anterior end of which is surrounded by a fringe of cilia. After a motile period these attach themselves by the anterior end to some firm body, and, forming a cell-wall, develop into new filaments. Note young plants in early stages of germination: they may be found in numbers attached to submerged plants or stones, in waters where *Œdogonium* grows.

III. There is some variety in the details of development of the sexual organs in different species of *Œdogonium:* some species are monœcious, others dicœcious.

The **oogonia,** or female organs, are most easily observed, being spherically enlarged cells of the filament, borne singly or several together: in such oogonia note—

i. The rupture of the cell-wall at the period of maturity by a transverse slit.

ii. The beak-like canal, which projects in some species from the slit.

iii. The hyaline receptive spot.

iv. In old oogonia the mature **oospore** with thick wall and dense contents.

The **antheridia,** which are smaller and shorter cells than those of the normal filament: each divides into two cells, the contents of which, without further division, escape as a motile yellow **antherozoid** similar in form to the swarm-spore. Attempts should also be made to observe the germination of the oospore.

With *Œdogonium* it will be well to compare species of *Bulbochæte*, which resembles *Œdogonium* in its fresh-water habit, its mode of attachment, and in its processes of cell-division and reproduction; but differs from it in its profuse branching, and in the presence of peculiar long bristles with swollen bases, which are borne on the ends of the branches.

ULOTHRIX ZONATA, *Ktz.*

This Alga is to be found, especially in spring and early summer, in slow-flowing streams, and ponds of fresh water, or in cattle troughs, and fountains: it occurs attached to stones and other objects near to the surface, and is to be recognised as a delicate, filamentous, unbranched organism of a bright green colour: it is attached by an attenuated base to the substratum, and the simple filaments are composed of numerous cells, each having a median zone-like chromatophore; hence the specific name.

Mount some filaments of the Alga in fresh water, and cover with a glass slip: neglecting for the present those specimens in which the reproductive processes

are going forward, note in those which are growing in a vegetative manner the following structural points :—

1. The cylindrical unbranched form of the Alga.

2. The **septa,** dividing the filament into a number of short cylindrical, or slightly barrel-shaped cells, of more or less unequal length. Note cases where cell-division is in progress, or only recently completed.

3. The **base of attachment,** which is usually attenuated, colourless, and often branched so as to obtain a hold on the substratum: granules of soil, &c., will usually be found attached to it.

4. The smooth **cell-walls,** consisting of a thin, outer, mucilaginous layer, and an inner, denser layer immediately surrounding the protoplasm.

5. The **protoplasmic body,** in which will be found—

a. A single **nucleus,** in a lateral position, and more easily recognised after staining with an iodine solution.

b. A median zone-like chlorophyll body, or **chromatophore,** which has the form of a flattened but divided ring.

c. A central vacuole.

Associated with *Ulothrix* will often be found the following Algæ which are allied to it: viz. *Stigeoclonium,* of which the filaments are similar to those of *Ulothrix,* but differ in being profusely branched; and *Chætophora,* which also consists of branched filaments, but they are connected by their swollen cell-walls into a more or less spherical gelatinous mass.

The reproductive processes are best seen in spring or early summer, and are encouraged by a gentle rise of temperature: they may be best observed in material

brought from outside in the morning into the higher temperature of the laboratory or working-room. There are two modes of reproduction of this Alga by means of **zoospores,** and they are readily distinguished by the size of the motile cells. Thus there are to be recognised—

1. The **macro-zoospores**, or asexual reproductive cells: these are relatively large, spherical or pear-shaped, green, primordial cells, furnished with four cilia attached to the hyaline anterior end, in which there is also a contractile vacuole. According to the bulk of the filament, one, two, four, or perhaps sometimes eight of these may be produced from a single cell: observe how they escape by a lateral hole in the cell-wall, being surrounded for a time by a membrane from which they soon free themselves, and begin active movement: finally they settle, form a cell-wall, and germinate directly to form new individuals.

2. The **micro-zoospores** or sexual gametes: these are relatively small; and according to the bulk of the filament, eight, sixteen, or thirty-two of them may be produced from each cell: observe how they also, after passing through a lateral hole in the cell-wall, are at first enveloped in a thin membrane, which is soon burst, and they escape as small pear-shaped motile bodies with two cilia. Observe carefully how these **conjugate** in pairs, coalescing first by their hyaline ends, then along their whole length; the resulting **zygospore** still continues to move by its four cilia for a time after conjugation, but finally settles down, and forms a cell-wall in about two days.

It will be difficult to observe the further growth

and development of the zygospore, since it undergoes a period of rest during the summer months, and its progress is very slow during the succeeding winter.

Preparations showing active zoospores should be treated with iodine solution, by which they will be killed, and the observation of the **cilia** will be easy by reason of the staining: note the four cilia in the macro-zoospores, while there are only two in those micro-zoospores which have not conjugated.

Trace the processes of change in the cells of the filament preceding the escape of the zoospores: according to Dodel and others their formation is by a process of cell-division: Strasburger, however, states that the process is simultaneous; being a case of free cell-formation, preceded, however, by a process of nuclear division (see *Zellbildung*, 1880, page 75).

SIPHONEÆ.

VAUCHERIA SESSILIS, *Vauch.*

I. This Alga is to be found growing as a lax green felt on the surface of moist soil (frequently on the soil in pots in greenhouses): it is of so coarse texture that the separate filaments can readily be recognised with the naked eye, having a somewhat dull, green, glassy appearance. Remove a small portion of this felt: tease it out as gently as possible in water, and examine it under a low power: observe—

1. The coarse, green, cylindrical tubes which constitute this Alga.

2. The absence of septa as a rule, though septa may be present occasionally in unhealthy specimens, and are formed in connexion with the reproductive processes.

3. The very irregular, and far from frequent, monopodial branching, and the rounded ends of the filaments.

4. Some branches may develop as **rhizoids,** ramifying in the soil, but these are frequently absent altogether.

5. There may be present lateral outgrowths of

peculiar form, which are the organs of sexual reproduction (**gametangia**), viz.—

a. Curved cylindrical bodies, which are the **antheridia.**

b. Obliquely oval, sessile bodies, which are the **oogonia.**

In this species the sexual organs are associated together in groups of two or three, each being inserted separately upon the thallus: a single antheridium is usually associated with one or two oogonia.

It will be necessary to distinguish the species above named both from other organisms not nearly related to it and also from other species of the same genus. Thus, the Moss protonema is usually found growing on the surface of pots, but it is readily distinguishable by its septa and mode of branching: other septate filamentous Algæ may also be associated with it, but are easily distinguished by their size, colour, septa, &c. Of other species of the genus *Vaucheria*, that most likely to be present is *V. terrestris*, which may be distinguished from *V. sessilis* by the antheridia and oogonia being inserted together on a common pedicel: other species differ in the insertion, number, and form of the antheridia and oogonia.

II. Put on a high power, and examine the structure of the thallus in detail. Note—

1. The smooth continuous external **cell-wall:** this may be made more apparent by plasmolysing some filaments with a 2 per cent. solution of common salt.

2. The **protoplasmic membrane,** which lines the wall, and incloses a large central vacuole which runs the whole length of the filament: this membrane may also be more readily distinguished in plasmolysed specimens. In the membrane are embedded—

3. Numerous oval, or spindle-shaped **chlorophyll**

corpuscles: look for some of these undergoing division.

4. Round highly refractive **oil globules,** which are more or less numerous according to the condition of the plant as regards nutrition.

In addition to the above bodies embedded in the protoplasmic membrane, numerous small bodies, which have the characters of nuclei, are to be found; but in order to see them careful staining is necessary, and the following method is recommended for this purpose: treat fresh specimens with a deeply coloured solution of methyl-green in 1 per cent. acetic acid: wash quickly with 1 per cent. acetic acid, and mount in the same. Examination under a high power will show the presence of numerous small, stained bodies lying in the stratum of protoplasm immediately within that containing the chlorophyll corpuscles: on the ground of their staining reactions these are described as **nuclei:** the most satisfactory results are to be obtained in filaments in which the chlorophyll corpuscles are not densely crowded, or from the examination of the ends of growing filaments. The specimens may be permanently mounted in a mixture of weak glycerine and acetic acid.

III. On specimens which have been kept under conditions favourable for strong growth, the sexual organs (**gametangia**) are usually to be found in greater or less numbers. Having found a specimen with mature sexual organs, examine first the **antheridium** under a high power.

1. The lower straight portion, or pedicel, rises vertically from the main filament, its contents are in direct communication with those of the filament, but are separated from those of the antheridium proper by a transverse septum.

2. The curved portion, or **antheridium** proper, differs from the pedicel in the contents being for the

most part colourless: when mature the contained protoplasm forms a large number of small **antherozoids,** which escape through an opening which appears at the apex.

By a comparison of a number of specimens various stages of development of the antheridium may be observed: *e.g.* (1) its origin as a rounded papilla from the main filament; (2) the appearance of the septum; (3) the opening of the pore at the apex, and escape of the antherozoids; (4) the empty wall of the antheridium with the pore at the apex through which the antherozoids have escaped; (5) the form of the antherozoids and their cilia are to be observed in specimens stained with iodine solution.

Next examine a mature **oogonium** in detail: note its sessile position, and the septum which separates it from the main filament: its oblique form and green-coloured, granular contents: when actually mature an obliquely lateral beak is formed, the apex of which becomes gelatinous, so that the motile antherozoids can gain access to the protoplasm.

By comparing specimens of different ages the following points may be observed: (1) the origin of the oogonium as a rounded outgrowth from the main filament; (2) the appearance of the septum; (3) the opening of the apex of the beak, and extrusion of a portion of the protoplasmic body, leaving exposed the colourless receptive spot; (4) attempts should be made to observe the actual entry of the antherozoids.

It is stated that the development of the sexual organs takes place at night, and the process of fertilisation during the day, the whole being completed within the twenty-four hours.

IV. Observe the changes which succeed fertilisation, resulting in the formation of the ripe **oospore:** the chief are—

1. Formation of a firm wall completely surrounding

the fertilised oosphere, and fitting closely within the wall of the oogonium.

2. An increase in the size and number of the oil globules.

V. The reproduction by means of zoogonidia may readily be observed in specimens grown under favourable conditions in water: a considerable mass of the Alga is to be placed in a porcelain bowl, in water, and exposed in a window: after a few days, numerous small plants of *Vaucheria* will be found floating on the surface of the water, or disposed along the submerged surface of the bowl: these have resulted from vegetative reproduction by **zoogonidia.** In order to see the process observations must be made in the early morning, or else the culture must be kept in the dark till shortly before the observations are to be made. Shortly after dawn (or soon after the specimens have been exposed to light) some filaments may be seen with a lens to have dark-coloured and slightly swollen ends: these are about to form zoogonidia: mount some specimens without a cover-slip, taking care to avoid injuring them; and examine under a low power: observe—

1. The swollen end of the filament, with dark, densely aggregated protoplasm, surrounding a vacuole.

2. The transverse **septum** dividing the swollen end from the rest of the filament.

If such specimens be kept under observation the escape of the zoogonidium may be observed: the following points are to be specially noted—

1. Various changes in the protoplasmic body and vacuole, terminating in the formation of a transparent, and radially striated, outer protoplasmic coat (**ecto-**

plasm), which lines the cell-wall, while darker, more granular protoplasm (**endoplasm**), including the chlorophyll corpuscles, collects towards the centre.

2. The rupture of the cell-wall by an irregular slit near to the extreme apex of the filament: before the rupture the septum may be seen to present a convex surface to the rest of the filament, indicating greater internal tension in the "zoosporangium": on rupture this is relieved, and the septum then projects convexly into the cavity of the empty zoosporangium.

3. The passage of the protoplasmic mass through the opening, by a streaming movement, assisted by more or less marked, screw-like rotation of the whole body: the protoplasm may undergo division at the time of escape, and two zoogonidia may thus be formed.

4. The rapid movement of the large zoogonidium when free, which may be followed with the naked eye, and under the microscope is seen to be rotatory. The motile period lasts but a short time, and varies in different species.

Treat a zoogonidium, which has just escaped and is in rapid motion, with a solution of iodine: put on a cover-slip, and examine under a high power: no cell-wall will be visible, though the solution will in some measure plasmolyse the cell. Turning more especially to the ectoplasm, there will be seen numerous **cilia,** arranged in pairs, projecting from the surface of the zoogonidium, while in the transparent ectoplasm will be seen numerous highly refractive bodies, which stain with iodine: these are **nuclei,** and a careful observation will show that their position is exactly opposite the insertion of the pairs of cilia.

This method of demonstration of the nuclei has the advantage of simplicity, but the results are not permanent; if it be desired to obtain specimens for keeping, they should be stained, as above directed, with methyl-green and 1 per cent. acetic acid; or better with hæmatoxylin.

Treat a zoogonidium, which has come to rest, with a plasmolysing agent such as 2 per cent. solution of common salt, watching it meanwhile under a high power: the protoplasm will contract, and a fine **cell-wall** will be seen. This result may also be obtained by pressure on the cover-slip.

Mount and examine zoogonidia which have already come to rest, and by a comparison of them the process of germination of the zoogonidia, and the development of new plants from them may be deduced.

For comparison with *Vaucheria* another member of the Siphoneæ may be examined, viz. *Codium tomentosum*, which is to be found growing near low-water mark on rocky shores, and is an Alga of very wide distribution, though not of very general occurrence on the British coasts.

Observe externally the cylindrical, green, spongy thallus, which branches dichotomously, and is attached to the rocky substratum by an extended disk.

Transverse sections should be cut: if fresh material be used, they may be mounted in sea-water; if alcohol material, in weak glycerine. Note, under a low power, the central felt of interwoven tubes, which are relatively narrow, and occasionally septate: the peripheral part of the section is occupied by a band of larger elongated sacs, arranged in a radiating manner.

Tease out a small piece of the thallus gently with needles: it will then be seen that the thallus consists of a system of branched tubular sacs, similar to those of *Vaucheria*, but aggregated together to form a **spurious tissue**, similar to that of the larger Fungi.

If the material be suitable, the sexual organs (**gametangia**) are to be found as short cysts, borne as lateral branches among the radiating peripheral sacs of the thallus, and completely overtopped by them. According to their size, smaller male (**antheridia**) and larger female (**oogonia**) are to be recognised on different individuals.

Observations may also be made on specimens of the native genus *Bryopsis*.

If specimens be at hand, comparison should also be made of various species of *Caulerpa*, in which the thallus attains high complexity and varied conformation, though it is not partitioned into cells: in this genus reproductive organs have not yet been observed.

Acetabularia, which is a native of southern seas, and *Botrydium*, which is to be found growing on moist soil in swampy places, may also be examined with advantage if material can be obtained.

VOLVOCINEÆ.

VOLVOX GLOBATOR.

This Alga is to be found swimming freely in fresh-water ponds, mostly in exposed situations: it is of such a size that its simple spherical body can be recognised with the naked eye.

Mount one or more in water in a hollow-ground slide without a cover-slip, and examine under a low power: observe—

1. The hollow spherical form of the whole organism (**cœnobium**).

2. Its rotating movements in the water.

3. The tissue of the hollow sphere, consisting of numerous cells forming a single layer, and for the most part similar to one another.

4. The **daughter-cœnobia** of various sizes in different specimens: their number is usually **eight.** They may be seen projecting into, or even almost filling, the cavity of the parent.

Mount a cœnobium in water under a cover-slip, and examine it in detail under a high power: it will of course be squeezed flat by pressure of the cover-slip: in the tissue thus flattened observe—

1. The ordinary cells of the cœnobium, each consisting of a protoplasmic body with green **chromatophore,** a

red spot, and a slowly pulsating vacuole: each cell is furnished with two **cilia,** which project beyond the surface of the cœnobium: these are better seen after staining with iodine.

2. The fine threads of protoplasm, also seen more distinctly after staining, which traverse the swollen cell-walls, and connect the cells together as a continuous network.

3. At the extreme outer surface of the cœnobium note a more clearly defined membrane, marked off into polygonal areas corresponding to the cells: in this is the swollen gelatinous substance which fills the whole cavity of the cœnobium.

4. The cells which form the **daughter-cœnobia:** these may be recognised as of larger size than the rest, the protoplasm being denser. Observe in different specimens their various stages of division, first into four, then into eight cells: the disk-like group thus formed becoming convex (by reason of quicker surface-growth at the centre than at the periphery), and gradually assuming the form of a hollow sphere, which projects into the cavity of the parent: the **pore,** corresponding to the margin of the original disk, may be seen after the young cœnobium has assumed the spherical form, and even when it has attained a considerable size.

During the summer nothing further will be observed as to the mode of reproduction of *Volvox* beyond that vegetative propagation above described, which is repeated through a series of generations; but in the autumn **sexual organs** may be formed, the **antheridia** and **oogonia** being borne on the same individual in *V. globator*, and are easily distinguished from one another, and from the neutral cells of the cœnobium.

i. The **antheridia** are to be recognised as enlarged cells with

disk-shaped contents; the disk breaks up into a large number of rod-like bodies arranged like a bundle of cigars. These may, under favourable circumstances, be seen to separate, and escape from the antheridium into the cavity of the cœnobium as spindle-shaped **antherozoids** having two cilia attached laterally.

ii. The **oogonia** are easily recognised as enlarged flask-shaped cells which project into the cavity of the cœnobium.

iii. The result of fertilisation is the **oospore**: these may be seen in considerable numbers in a single cœnobium: they are of spherical form, and show a thick cell-wall, developed inside that of the oogonium: the wall consists of an **epispore**, a firm membrane, with numerous superficial spines, and an **endospore**, which is a smooth layer. The germination has been observed in *V. minor* to result in the formation of a new cœnobium by a process similar to that in the vegetative reproduction: the same is presumably the case in *V. globator*.

If material be at hand, observations should also be made on *Pandorina*, the freely-swimming cœnobium of which is simpler in structure than that of *Volvox*, consisting only of sixteen cells. The sexual process consists in the conjugation of two freely-swimming, and similar swarm-cells.

PLEUROCOCCUS VULGARIS, *Meneg.*

This is the organism which is universally to be found forming a bright green pulverulent incrustation on the bark of trees, wooden rails, &c. If a small quantity of it be mounted in water, and examined under a low power, it will be seen to consist of cells with green-coloured contents; occurring sometimes solitarily, but more frequently in groups of two, or four, or even larger numbers. Examination under a higher power will show that they multiply by division, and that the resulting cells tend to round themselves off. Note especially that in groups of four or more cells, a splitting of the cell-wall at the centre of the group

is frequently to be observed, which is doubtless the result of the tendency of the cells to assume a spherical form.

Select a single large cell for examination under the highest power, and observe the following details—

1. The **cell-wall** is thick, and shows clearly a double contour: staining with Schulze's solution gives a blue coloration of the cell-wall.

2. The protoplasmic contents, which at first sight appear uniformly green, will show differentiation, especially after staining with iodine solution: thus there may be recognised—

a. A **nucleus,** which is usually central, though sometimes it is in a lateral position.

b. **Chromatophores,** a number of which together form a partial envelope surrounding the nucleus: there are no pyrenoids.

c. A very scanty colourless **protoplasmic basis,** in which these bodies are embedded.

It is held that *Pleurococcus* is the resting stage of *Chlamydomonas*, an organism which differs from other Volvocineæ in that its motile cells are separate, and do not form a cœnobium. Material of *Pleurococcus* should be kept in water exposed to light, and observations made from time to time to see the motile cells, with their limiting cell-wall and active cilia: the latter are best seen after staining with iodine solution.

Hydrodictyon utriculatum, *Roth.*

Observations may with advantage be made on *Hydrodictyon*, because of the very peculiar formation of the tissue composing its network. It is not, however, of very frequent occurrence: it is

to be found floating as a hollow sac-like net in still, fresh water in summer. Observation with the naked eye will show obviously the meshes of the network.

Mount a small piece in water and examine under a low power: the four- to six-angled meshes will be seen to be limited by as many cells, which are of large size. Each cell contains numerous nuclei. When a new network is to be formed, the protoplasm of one of these cells divides simultaneously into 7,000 to 20,000, which move about, within the original cell-wall, and finally arrange themselves in a new network, which, on growing, is liberated by rupture of the parent cell-wall. This process may be observed in healthy specimens in the height of summer.

The whole cycle of life is a complicated one, and is somewhat difficult of observation.

CONJUGATÆ.

SPIROGYRA.

I. In summer there may frequently be found, in stagnant or slowly-flowing waters, flocculent freely-floating masses of a vivid green colour, and slimy to the touch: with the naked eye it may be seen that the masses consist of coiled and tangled unbranched filaments, in which there is no distinction of apex and base.

Mount a few of them in water, and examine them under a low power: note that the simple unbranched filaments are partitioned off by transverse septa into a number of relatively short cells. It will usually be obvious that the filaments are not all alike, and two chief types will frequently be found present—

a. Those with two star-like green bodies in each cell: these belong to the genus *Zygnema*, and, as these Algæ are not so well fitted for a detailed observation, they may be neglected.

b. Others will be seen to have one or more spirally coiled green bands in each cell: these belong to the genus *Spirogyra*.

A superficial observation of specimens collected at the same time and place will usually show that in

different filaments there is considerable variety in size, form of the cells, thickness of the walls, and in the number and arrangement of the spirals: according to these characters (together with those of the zygospore) a large number of species of *Spirogyra* are distinguished. It will be found convenient to select for observation specimens of the largest size, and with the coils of the green spirals furthest apart. Examine such filaments in detail under a high power, in the living state, mounted in water, and observe—

1. That the whole filament is covered externally by a transparent **gelatinous sheath,** with a somewhat irregular outer surface, and showing a radial striation: this is stated by Klebs to be an excretion from the protoplasm, not a result of metamorphosis of the outer layer of the cell-wall. It is to this layer that the Alga owes its slimy character.

2. A firm **cell-wall,** which is more highly refractive: it immediately surrounds the protoplasmic body, and is continuous with the transverse **septa.**

In respect of the nature of the septa there is some variety in different species, and the whole genus may accordingly be divided into two sections: (i.) those in which the septa appear as simple lamellæ, and the ends of the cells are then truncate; (ii.) those in which the septum is split in the central part of its area into two layers, which separate from one another and appear as two involutions, encroaching on the cavity of the cells. A sort of vegetative multiplication of these plants takes place by breaking up of the filaments, accompanied by splitting of the septa: in the species of section (ii.) the involute walls are then pressed outwards by the turgescence of the cells, and become convex: this may often be observed by moving the cover-slip while gently pressing on the object.

3. The **protoplasmic body,** which consists of—

a. A colourless membrane (**primordial utricle**) which lines the cell-wall internally, and surrounds the large central **vacuole.**

b. The green spiral **chromatophores** (one or more), embedded in the membrane: note their irregular outline, and the numerous highly refractive lenticular bodies (**pyrenoids**) which are contained in them.

c. A bi-convex lens-shaped **nucleus,** suspended in the centre of the vacuole by fine colourless strands of protoplasm, which run to the primordial utricle, attaching themselves to points opposite the pyrenoids.

Stain with an iodine solution, and observe that the colourless protoplasm stains pale yellow, the nucleus a deeper yellow, and it will thus be more clearly seen, as well as one or sometimes two **nucleoli,** which are deeply stained: the pyrenoids stain a dusky purple.

A careful comparison of these pyrenoids under high powers, with and without iodine staining, will lead to the conclusion that they are highly refractive, colourless bodies, around which is usually present a coating of starch, either as a continuous sheath, or in distinct granules. Such bodies are to be found in the chromatophores of many Algæ. Look for examples illustrating their multiplication by fission. In material decolorised in alcohol, or fixed by some other method, apply such staining reagents as hæmatoxylin and carmine: the pyrenoids will stain in a manner similar to nuclei.

II. The process of cell-division may be very well observed in the filaments of *Spirogyra;* the chief difficulty is however that the process normally takes place at night, beginning about 10 to 12 P.M. Strasburger, who has made this a subject of careful

study, recommends that the *Spirogyra* be placed in a flat plate upon a block of ice during the night: if on the following morning the plants be exposed to a higher temperature, the cell-division which had been previously retarded will begin, and the successive stages may be followed.

Though the main points in the process of division may be observed in fresh material, the minute details will be better seen in material which has been fixed in 1 per cent. chromic acid for about four hours, or in picric acid: after washing carefully, the material may be stained with carmine or hæmatoxylin, and mounted in very weak glycerine.

The following points in the process should be specially noted—

1. The disappearance of the nucleolus.
2. The formation of the striated nuclear spindle and of the equatorial nuclear disk.
3. Division of this, and collection of the two halves at the poles of the spindle as the new nuclei, which are still connected by fine threads.
4. The subsequent dilatation of the spindle, the threads becoming more curved.
5. Meanwhile the collection of microsomata at the equator of the dividing cell.
6. The involution of the chromatophores and their subsequent division.
7. The gradual formation of the septum, beginning at the periphery and proceeding towards the centre.

III. In summer or autumn the process of **conjugation** and formation of the zygospores may frequently be observed in *Spirogyra*: filaments which are about to

conjugate assume a position parallel to one another, and on them the following observations are to be made—

1. Cells opposite one another put out rounded processes which meet.

2. The wall at the point of junction is absorbed, and thus the canal of communication is formed.

3. Meanwhile the protoplasm of the two cells has rounded off, one (the male) usually doing so earlier than the other (the female).

4. The protoplasm of the male cell passes through the canal, and coalesces with the female to form the **zygote.**

5. The zygote surrounds itself with a thick stratified cell-wall, which is smooth or shows various markings of the surface according to species.

By fixing material in course of conjugation and staining with hæmatoxylin, Schmitz has been able to observe that the nuclei of the conjugating cells coalesce to form the single nucleus of the zygospore.

For comparison with *Spirogyra* observations should be made on *Mesocarpus*, a filamentous fresh-water Alga, having in each cell a single straight flattened chromatophore, in a central position. The conjugation differs from that of *Spirogyra* in two points: (1) that the zygospore is formed in the conjugation-canal, and not in the cavity of one of the conjugating cells; (2) that a part only of the protoplasm of the conjugating cells is used up in forming the zygospore.

DESMIDIEÆ.

Members of the closely-allied group of the *Desmidieæ* should also be examined; *e.g.* species of *Closterium*,

which is not uncommonly to be found in stagnant water. Mount specimens in fresh water, and examine under a high power: note—

1. The more or less semilunar curved form of this unicellular Alga.

2. The smooth cell-wall, often marked by transverse striæ, which are indications of former cell-divisions.

3. The colourless protoplasmic basis, which includes—

   a. Two large **chromatophores** of equal size, disposed symmetrically on either side of a central clear space.

   b. A single **nucleus,** which occupies the central space, but is sometimes difficult to observe owing to the fact that the chromatophores often extend across the central space, and may even be connected.

Observe further the peculiar plaited form of the chromatophores, which also include several disk-shaped, highly refractive bodies (**pyrenoids**). Stain with an iodine solution: this will make the nucleus more apparent, and the pyrenoids will stain a dusky purple.

The plant multiplies by cell-division: try to observe cells in which the process is going on. It also conjugates, though this will not be readily observed.

Other Desmids may also be compared, *e.g. Micrasterias*, which is to be found in peaty pools: it is of flattened form with a deep median constriction, and stellate outline: the pyrenoids are very well seen in this species. For description of other forms of this family reference must be made to books dealing specially with the subject.

Observations should also be made on some of the *Diatomaceæ*, a family of somewhat doubtful affinity, and remarkable for the variety and beauty of form of its members.

Almost any sediment from a fresh-water pool, mounted on a slide and examined under a high power, will show specimens of *Navicula:* it is to be recognised as a unicellular organism pointed at both ends, and showing active movement. Note the yellowish brown colour characteristic of Diatoms, and observe carefully that within the cell-wall there is a basis of colourless protoplasm, in which are embedded a nucleus, and a yellowish brown coloured chromatophore, of variable shape and position. Try to find examples of cell-division, which takes place longitudinally, and observe the details of the process.

Specimens of *Pinnularia*, which is also a common form, should be observed, since in it the peculiar structure of the cell-wall is to be seen: it is composed of two halves, which fit one inside the other like a pill-box and its lid.

A third form, *Gomphonema*, is to be found attached by a thin transparent stalk to the surface of almost any submerged plant: in this the characters above noted may be again seen.

The formation of **auxospores** may be observed in these or other forms, especially in summer. Attention is to be paid to the varieties of surface-marking in the cell-walls of these and other Diatoms: for further details reference is to be made to special treatises.

By treatment with a little potassium chlorate and nitric acid, and subsequent ignition on platinum foil, skeletons of silica may be prepared, which retain in minutest detail the configuration, and surface-markings of the "tests" of the Diatoms so treated.

CYANOPHYCEÆ.

NOSTOC.

This plant is to be found growing on turf, or Moss, but not attached to it. It appears as irregular, more or less flattened, olive-green masses, which are brittle when dry, but soft and gelatinous when wetted.

Mount a small piece, or a section of one, in water, and it will be seen under a low power to consist of numerous irregularly coiled filaments, embedded in a gelatinous matrix.

Under a high power each filament may be seen to consist of series of small cells, with granular, blue-green contents, which constitute the great proportion of the filaments; these series are interrupted here and there by larger cells with firm, clearly-marked walls, and transparent contents: these are the **heterocysts.** Observe cases of cells which have recently undergone division. Attempts may be made, by cultivating *Nostoc* in water, to observe the separation and further development of new masses by means of the **hormogonia** (cf. Goebel, *Outlines*, Eng. Ed., page 22).

Compare the above observations with those on *Collema* (page 117): it is to be noted that *Nostoc* and *Collema* are frequently to be found closely associated

together, so that it is difficult to tell where the Alga ends and the Lichen begins.

OSCILLATORIA.

Organisms belonging to this genus are to be found as dark blue-green, olive-green, or black coverings on damp walls where water is constantly trickling, on wet soil, &c. Mount a small quantity in water, and examine under a high power: they will appear as fine cylindrical, unbranched filaments, with very delicate cell-walls, and cell-contents of various blue, green, and olive tints. Note the septa dividing the whole into disk-shaped cells: also the convex ends, and the constant oscillating movements, from which they derive their name.

Staining with the usual reagents will disclose no nuclei, though minute granules may be stained.

GLŒOCAPSA.

This organism may be found, usually associated with others, in those slimy masses which frequently grow on the inside of the glass in hot-houses. Examine it under the microscope, and note the numerous cells with pinkish cell-contents, and much swollen cell-walls. The cells will usually be associated in groups, which, from the course of the lines of stratification of the cell-walls, may be seen to have had a common origin.

The slimy masses of *Glœocapsa* will rarely be found to consist of it alone; other organisms being almost always mixed with it.

FUNGI.

I. BASIDIOMYCETES.

AGARICUS CAMPESTRIS, *L.* (The Common Mushroom).

I. Examine a brick of "mushroom spawn," such as is sold in the shops for the artificial culture of the Mushroom (*Agaricus campestris*). It will be found to consist of a compost of dried cows' dung, loam, and clay, in which numerous very fine microscopic filaments are present, or irregularly branched white bands which may be easily recognised with the naked eye. This is the **mycelium.** The best spawn is that in which the mycelium is generally distributed, so as to give the whole mass a uniformly grey appearance. It is to be remembered that the mycelium of other Fungi may, and most probably will, be present in greater or less amount.

Tease out with needles in water some of the mycelium, mount in water, and examine under a low power: note that the white bands recognised with the naked eye are composed of numerous colourless filaments (**mycelial hyphæ**), associated together in a parallel course, while

here and there single hyphæ diverge from the rest, and ramify through the compost.

II. In order to obtain an actively growing mycelium, bearing "mushrooms," the brick is to be broken into pieces, and these must be buried a few inches deep in a compost of similar nature to that of the bricks: the whole is to be kept moist, at a moderately high temperature, and in the dark: the stoke-hole of a conservatory is well adapted for the cultivation of mushrooms. After a period of a few weeks, the compost will be found to be permeated by a mycelium, similar to that in the brick of "spawn," while numerous mushrooms of various size will be found connected with it: such a culture as this will suffice for the study of *Agaricus campestris* in the laboratory.

III. Remove a small piece of the mycelium of an actively growing culture, mount it in water, and observe under a low power that it is similar in its main characteristics to that in the dormant state in the brick of spawn.

Having teased it out carefully with needles, examine it in detail under a high power, and observe—

1. The **hyphæ,** of cylindrical form, and with rounded free ends.

2. The irregular **branching** of the hyphæ.

3. The **septa,** which are transverse, and situated at irregular intervals.

4. Hyphæ may frequently be seen to be incrusted by numerous **rod-like crystals:** these are especially numerous in the dormant mycelium: it is to this, in great measure, that the mycelium owes its chalky white appearance.

By applying the ordinary tests, it may be shown that the incrustation consists of calcium oxalate.

IV. Examine a portion of the mycelium which has begun to produce "mushrooms": with a little care the compost may be entirely removed from considerable tracts of the branched mycelium, and then the relation of the latter to the young mushrooms may be clearly seen. If a series of specimens illustrating the development of the mushroom be examined with the naked eye, the following observations may be made—

1. That the mushrooms arise from the mycelium itself.

2. That they appear first as irregular rounded, or oval, upward growths, of denser texture than the mycelium itself.

3. That on cutting one of the smaller mushrooms longitudinally it appears to the naked eye to be of homogeneous structure.

4. That older mushrooms acquire an enlarged head (the **pileus**), which is supported on a cylindrical stalk (the **stipe**). In this state they are termed "button mushrooms."

5. That as the pileus dilates horizontally, a rupture of a veil of tissue (**velum partiale**) about its lower margin exposes a complicated laminated structure formed internally (the **gills,** or **hymenial lamellæ**).

6. Note further the ring or **annulus,** which remains persistent on the stipe of the mature mushroom, and marks the line of rupture of the velum: the corresponding, irregular fringe at the margin of the pileus is also to be recognised.

7. Removing the mature pileus, examine its lower

surface, and note the radiating, more or less darkly coloured **lamellæ,** some of which extend the whole way from the margin to the insertion of the stipe, others only a part of that distance.

Lay the pileus of a mature mushroom with its lamellæ downwards on a sheet of white paper for a few hours: on removing it there will be seen on the paper a sort of print of the configuration of the under surface of the pileus, produced by the fall of the minute, dark-coloured **spores:** the white lines of the print correspond to the spaces between the lamellæ.

V. In order to study the structure of the mushroom by means of sections, it is a great advantage to harden the material, and the following treatment has been found to produce good results: treat the fresh material for about twenty-four hours with 1 to ·5 per cent. chromic acid; wash with water, and then successively with 50, 70, and 90 per cent. methylated spirit: the tissues will assume a cartilaginous character, which makes it possible to cut fine sections: in preparing large specimens it is an advantage to cut them up into pieces of moderate size, so that the reagents may gain more ready access to the internal parts.

From material thus treated cut longitudinal sections of the stipe so as to include both peripheral and central tissues: mount in glycerine, and examine first with a low power: observe—

1. The whole is a **spurious tissue**, composed of elongated septate tubes (**hyphæ**), which are closely interwoven.

2. The diameter of the individual hyphæ is less, and

they are more closely packed towards the periphery than near the centre of the section.

Examining the sections under a high power it will further be observed—

1. That the hyphæ are branched, while occasionally their endings are to be seen.

2. That they are thin-walled, the transverse or oblique septa being so disposed that the cells are not much longer than broad.

3. Here and there are to be found hyphæ with fewer septa, and highly refractive contents.

4. The protoplasmic contents of the hyphæ which make up the bulk of the tissue are far from being copious, while no single, well-marked nucleus is to be found in the individual cells.

By careful staining it is possible to demonstrate the presence of numerous very small **nuclei** in the protoplasm of the hyphæ. Strasburger recommends *A. pratensis* as a more suitable object for this demonstration than *A. campestris*. If fresh material be used, the sections may be stained with methyl-green in 1 per cent. acetic acid, washed with 1 per cent. acetic acid, and mounted in weak glycerine and acetic acid; if chromic acid material be used, it should be carefully washed from the acid, and be stained with hæmatoxylin.

VI. Cut transverse sections of the stipe, and, treating as before, observe that the hyphæ appear circular in section, that they are more loosely packed towards the centre than at the periphery, and that throughout intercellular spaces are to be recognised.

Observe under a high power that where the section includes one of the septa (which will thus be seen in surface view) a central highly refractive spot is to be

seen, which may also be recognised in the septa in longitudinal sections, as a slight aggregation about the centre of the septum.

VII. Passing to the pileus of the mature mushroom, cut tangential vertical sections through it in such a way as to traverse the vertical gills at right angles to their surface: great care must be taken that the surfaces of the gills shall not be injured in the process of preparation, otherwise the basidia and spores which project from their surfaces cannot be observed. Mount in glycerine and examine under a low power: the chief bulk of the section will consist of the massive tissues of the pileus, which show little or no differentiation; passing downwards to the lower surface where the **gills** or **lamellæ** have been traversed, the sections of these will be seen as fringe-like projections from the lower surface: occasionally branching of the gill may be recognised.

Examine the sections in detail under a high power: the following observations are to be made—

1. The mass of tissue of the pileus consists of a complicated plexus of much-branched hyphæ, with large intervening spaces: it is composed of short cells, similar in their characteristics to those which compose the stipe: the chief difference lies in their arrangement. This spongy tissue becomes denser about the insertion of the lamellæ.

2. The sections through the lamellæ show a differentiation into—

a. The central portion (**trama**), in which the septate hyphal filaments are easily recognised running longitudinally down the middle of each lamella, and

curving outwards at their ends towards the free surface.

b. The **sub-hymenial layer,** composed of shorter, closely packed cells, constituting a pseudo-parenchyma: it may, however, be recognised, and especially in sections of young mushrooms, that this pseudo-parenchyma consists of the short-celled, terminal parts of the hyphal filaments which compose the trama.

c. The **hymenial layer,** consisting of oblong, closely packed cells, having their longer axes perpendicular to the outer surface: of these cells two types are to be distinguished—

i. The **paraphyses,** which are somewhat narrower and have smooth rounded ends.

ii. The **basidia,** which are more bulky, and longer: each bears on its end **two fine processes** (**sterigmata**); at the extreme tip of each of these there appears a swelling which develops into the mature **spore.** Note various stages of development of the sterigmata, and spores.

VIII. Remove a whole gill carefully from a fresh mushroom, mount it on a slide, without any reagent or cover-slip, and examine its surface with a medium power: it may then be seen that the dark colour is due to the dusky spores, which are thickly distributed over the surface of the gill in pairs, **two being produced from each basidium:** note further the pale colour of the tissue of the hymenium, and the rounded ends of the paraphyses, and of those basidia which are young, or have already produced mature spores.

A comparison may be made with *Coprinus micaceus:* this Fungus appears almost with certainty if cow-dung be kept for three or four weeks at a moderate temperature under a bell-glass: if sections be cut from the lamellæ, the structure of the hymenium will appear similar to that above described, but the number of sterigmata on each basidium is **four.** On species of *Coprinus* large bladder-like cells **(cystidia)** are found projecting from the hymenial layer.

Examine specimens of other Hymenomycetous Fungi, *e.g.* species of *Polyporus*, *Dædalea*, *Boletus*, and *Hydnum:* note in them the difference of conformation of the thallus, and especially of the hymenial surface; also the difference of texture: thus in *Dædalea quercina*, and in some species of *Polyporus*, the thallus is hard, and of a corky or woody nature, while in others (*e.g. Polyporus giganteus*) it is soft and succulent.

Sections should be cut from the thallus of *Dædalea quercina*, or some other woody form: an examination of them will show that, though the thallus is hard, the structure of it is similar to that of the mushroom, the whole being composed of branched septate hyphæ.

Sections should also be made through the hymenium of some species of *Polyporus* in such a way as to cut the pores transversely: examination of these will show the hymenial layer lining the pores, and consisting, as in the mushroom, of paraphyses and basidia, the latter producing sterigmata and spores; but in *Polyporus* the hymenial layer is less closely packed and regular.

A comparison should also be made of some types of the Gasteromycetes, *e.g. Lycoperdon*, *Geaster*, *Crucibulum*, and *Phallus.* In these forms it will be seen that there is considerable variety in detail of arrangement of the hyphæ, resulting in a marked difference of external conformation of the mature Fungus. Still, sections of them in relatively young stages will show that they also are composed of branched and septate hyphæ, while the mode of formation of the spores on the basidia of more or less regular **internal** hymenial layers, corresponds essentially to that already described for the mushroom.

II. ÆCIDIOMYCETES.

PUCCINIA GRAMINIS (Æcidium Berberidis), (Rust of Wheat).

A. *Puccinia Stage.*

I. There may often be found on the stems and leaves of wheat and others of the Gramineæ in winter, dark oblong patches, which owe their origin to a Fungus (*Puccinia graminis*) that infests the tissues, and produces the disease called *Rust.*

Examine one of these patches with a lens, and note that the superficial tissues of the wheat are ruptured by a longitudinal slit, and the torn edges are turned back, so as to expose a dense, dark-coloured mass, which protrudes from within: the nature of this mass must be studied by means of sections.

II. Cut transverse sections of the leaf-sheath, or other diseased part of the Grass plant, taking care that the section shall traverse one or more of the dark patches of Rust: mount in glycerine, and examine under a low power. Observe that the structure of the greater part of the section is normal (see Part I. page 114, &c.): the dark patches will be seen to be opposite one of the spaces between the vascular bundles, while the epider-

mis, which normally covers over the tissues, is ruptured. In case it is the leaf which has been cut, dark patches may be observed as rupturing and projecting through the epidermis of both the upper and lower surfaces.

Put on a high power, and in a thin section observe—

1. The thin **hyphæ** of the branched **mycelium** of the parasite (*Puccinia*), which ramify in the softer, succulent tissues, but do not as a rule attack the sclerenchyma, or vascular bundles: they may be traced up to the dark patches above noted.

2. The masses of dark brown **teleutospores** or **winter spores,** which are produced by this mycelium, each spore being borne on a thin pedicel: each consists of two cells, with thick walls, differentiated into two layers, the **exospore** and the **endospore.** In the protoplasmic contents of each cell is a clear spherical body, which may be the nucleus, but this is not certain.

III. If pieces of a Grass plant bearing teleutospores be kept in a moist atmosphere (on wet blotting-paper, under a bell-glass) in the spring-time, a fine, white, semi-transparent growth will be produced from the teleutospores: this is the **promycelium.** Remove some of these germinated teleutospores carefully with a needle, and mount in water: if this be done without injuring the promycelium, it will be seen under a high power that one or both of the cells of the teleutospore have put out a germinal tube (the **promycelium**) by rupture of the exospore, and protrusion of the endospore: this promycelium divides into four or five cells, each of which (excepting the basal one) produces a conical process (the **sterigma**): the end

of each of the sterigmata swells into a small irregularly roundish body (the **sporidium**), which ultimately becomes detached.

By the above simple method of preparation the promycelium is apt to be damaged; it is much better to remove the teleutospores before germination, and to cultivate them in a hanging drop of water on the slide (see Part I. page 16). At the right period of the year, the germination takes place in about twenty-four hours: if this succeed, the additional advantage is gained from cultivation on the slide, that the observer will be able to follow the successive stages of the germination in an individual specimen, by repeated examination of it at short intervals of time.

It is known that the sporidia thus produced are not capable of further development on the Grass plant: this only takes place when they gain access to the Barberry (any species of *Berberis*), or other plants of allied genera, *e.g.*, *Mahonia*. In order to acquire evidence on this point, cultures should be prepared as follows; students must, however, be prepared for disappointment owing to various technical difficulties, which will only be duly appreciated in practice:—Keep fresh, young leaves of *Berberis* in moist air under a bell-glass, and in drops of water placed on the surface of them immerse some teleutospores which are known to be in a fit condition for germination: after an interval more than sufficient for complete formation of the sporidia (and De Bary states that infection may occur in twenty-four hours), strip off a small piece of the epidermis, or cut tangential sections from where the drop was placed: mount in water with the outer surface of the epidermis uppermost, taking care to avoid pressure by the cover-slip. Examine under a high power to observe the mode of germination of the sporidia, and the penetration of the germinal tube **through the outer wall** into a cell of the epidermis, whence it proceeds to the mesophyll, and, branching, spreads through considerable tracts of tissue: the result of this may now be studied in the mature condition on the leaf of *Berberis*.

B. *Æcidium Stage.*

IV. Note in early summer on the leaves of *Berberis* irregular bright yellow blotches, the tissues of the leaf appearing swollen at those spots, and projecting convexly on the lower surface, while the upper surface of the blotch is usually concave: on the lower surface numerous irregularly distributed yellow cups (**æcidium cups**) may be seen projecting slightly beyond the surface, while on the upper surface also may be seen projecting organs of smaller size, and irregular distribution (**spermogonia**).

V. Cut transverse sections of a diseased leaf, so as to pass through one of these blotches: mount some in glycerine, others in Schulze's solution, and examine under a low power: observe—

1. In the thinner **normal** part of the section, that between the upper and lower epidermal layers there is a mesophyll consisting of a single palisade layer, and five or six irregular layers of spongy parenchyma.

2. That the greater bulk of the **infected** part is due not so much to increased number of the cells as to the larger size of the individual cells and of the intercellular spaces.

In the sections prepared with Schulze's solution, if a good staining has been effected, note with a low power that the fungal tissues are but slightly stained yellow, while the tissues of the host are stained in the usual way, chiefly a dark blue. Recognise as the most prominent parts of the parasite—

1. The **æcidia,** cup-like structures, containing a

closely packed mass of **spores,** and opening by rupture through the lower epidermis of the host.

2. The **spermogonia,** relatively small, flask-shaped organs opening on the upper surface of the leaf.

Having thus gained a general idea of the sections, examine them in detail under a high power, and note that in the infested patch the cells of the host are apparently embedded in a felt of **mycelium,** consisting of septate and branched **hyphæ,** which traverse and completely choke up the intercellular spaces: they are but slightly stained with Schulze's solution, while the cell-walls of the host plant assume a dark colour: they are for the most part confined to the intercellular spaces, and especially those round about the æcidia; but it is stated that occasionally they penetrate the cells of the host, and though this is not easy to see, examples of it should be looked for. Turning to the æcidium observe—

1. Its cup-like form.

2. The dense felt of hyphæ at the base of it.

3. Immediately above this is the **hymenium,** a layer composed of closely packed, parallel, rod-like cells (**basidia**), arranged perpendicularly to the outer surface of the leaf.

4. The rows of **spores,** which have been successively abstricted from the basidia: observe the hexagonal form, thickened wall, and orange colour of the spores, and the way in which the spores of contiguous rows fit together.

5. The **peridium,** consisting of a single layer of cells enveloping the mass of spores: the form and arrangement of the cells resemble that of the spores themselves

though not so regular: note the thickened and striated outer wall.

Prepare similar sections from a young blotch, in which all the æcidia have not yet ruptured the epidermis of the host: and note—

1. The origin of the æcidia in the mesophyll of the host.
2. That the æcidia are relatively narrow when young, the hymenium growing broader as it grows older, by intercalation of new basidia.
3. The traces of displacement, and ultimately of rupture of the superficial tissues of the host.
4. The outgrowth of the peridium as a tube open at the apex, and extending beyond the general surface of the *Berberis* leaf.

Returning to the **spermogonia,** observe—

1. The closely packed, parallel, rod-like hyphæ converging to the centre (**sterigmata**).
2. The minute oval bodies (**spermatia**) abstricted from them, and escaping through the narrow pore on to the outer surface of the leaf.
3. The brush of hyphæ which protrude through the narrow pore.

A careful teasing out of the spermogonia with needles, and examination under a high power will be a profitable exercise. Attempts may also be made, with suitable precautions, to cultivate the spermatia in various nutritive solutions.

VI. It is known that the æcidium-spores of this fungus will not infect the Barberry plant afresh, but will only germinate so as to infect a Grass plant; thus the fungus is an example of "Heterœcism." The spores retain their germinating power only for a short period.

Take some fresh spores from an æcidium, and place them in a drop of water on the surface of a fresh leaf of some Gramineous plant: after keeping it in moist air for about 48–60 hours, strip off a part of the epidermis, or better, cut tangential sections of that part on which the spores have been placed: mount in water with the outer surface of the epidermis uppermost, and examine under a medium power: observe that the **æcidium spores** have produced tubular **hyphæ,** which make their way, **through the pores of the stomata,** into the tissues of the Grass plant.

VII. Infect a Grass plant with **æcidium spores** and keep it in a moist atmosphere: in about a week reddish swellings will appear about the points infected, and the epidermis will be ruptured.

Cut transverse sections so as to traverse one of these ruptured spots: mount in water, and observe under a medium power: note—

1. The branched mycelium ramifying in the tissue of the Grass.

2. The ruptured epidermis.

3. The closely packed **uredo-spores** of simple oval form, borne on thin pedicels (basidia). Observe further the **exospore,** rough with small outgrowths: the **endospore,** with four germinal pores, arranged equatorially; here the inner wall is wanting: note the protoplasmic contents with reddish granules.

Attempts should be made, as above directed for the æcidium spores, to infect leaves of the Barberry and of the Grass with these **uredo-spores,** when the infection will be found to succeed on the Grass, but not on the Barberry.

The infected Grass plants which have produced

uredo-spores should be kept till the autumn, when the patches which before produced uredo-spores only will, on investigation as above directed, be found to bear **teleutospores** intermixed with them, and finally to assume the winter condition of containing **teleuto-spores** or **winter-spores** only, in which condition the winter is passed: with this stage the study of the Fungus, as above directed, was begun.

III. ASCOMYCETES.

A. *DISCOMYCETES.*

PEZIZA.

For comparison on the one hand with *Claviceps* (p. 123) and *Eurotium* (p. 126) as members of the Ascomycetes, and on the other with the Lichens to be described below (p. 115), observations should be made on some species of *Peziza*. This is a very large genus, and the specific differences are somewhat difficult to master, but any species will serve the present purpose. It has been shown that some species at least have a gonidium-bearing stage, thus the mould known as *Botrytis cinerea*, which is common on decaying leaves, has been proved to be a stage in the life-history of *Peziza Fuckeliana*; but it is with the mature fructification or **apothecium** only that we shall now have to deal.

The flat or cup-shaped fructifications of *Peziza* are commonly to be found growing on decaying wood, &c., and vary greatly in size and colouring in different species. Having collected some, observe with the naked eye—

1. The smooth upper surface or **hymenium.**

2. The margin, which is hardly developed as a distinct excipulum.

3. The lower surface and base, which is usually covered with fine hyphal filaments, serving as rhizoids.

From material, either quite fresh, or hardened in alcohol, cut median vertical sections : mount in glycerine, and observe—

1. The large-celled pseudo-parenchyma about the lower surface, with the projecting rhizoid-like hyphæ which penetrate the substratum.

2. Passing upwards through the section the hyphal character becomes more obvious, the branched filaments forming a dense plexus.

3. The tissue again assumes more of a pseudo-parenchymatous nature in the **subhymenial layer.**

4. The **hymenium,** which is similar in its character to that of *Parmelia,* consisting of—

a. Elongated, narrow **paraphyses,** and—

b. Wider **asci,** each of which contains eight **spores.**

A careful comparison of various asci will give some idea of the mode of development of the spores by a process of free-cell formation.

Other forms of Ascomycetous Fungi may also be compared with advantage ; *e.g. Morchella* (the Morel).

B. *LICHENS.*

PARMELIA PARIETINA (Yellow Lichen).

I. This species is very commonly to be found growing on tree-trunks, walls, roofs, &c.: note its **foliaceous** thallus, which has no definite mode of branching, and is closely applied to the substratum. It is of a bright olive-yellow colour on the upper surface, but white underneath: note the processes from the under surface (**rhizines**) by which it is attached to the substratum also its brittle character when dry: soak it in water and it will become soft but leathery.

On the upper surface observe the more or less flattened or cup-shaped **apothecia,** which are of darker colour than the rest of the thallus, and attain a diameter of about one-eighth of an inch: note that those near the margin of the thallus are smaller than those nearer the centre.

II. From material which has been kept dry, select a small part of a thallus where no apothecia are present: soak it for a short time in alcohol, and embed in paraffin: cut as thin sections as possible: having allowed them to swell as much as they will in water, mount

them in glycerine, and examine under a low power: observe—

1. The irregular outline of the section.

2. The greater part of the thallus made up of colourless transparent tissue (**fungal tissue**).

3. The green **gonidial layer,** which is not very regular or sharply limited, and is situated at some distance below the upper surface of the thallus: since the gonidia are confined to a certain region of the thallus, and not distributed uniformly through it, this may be distinguished as a **heteromerous** Lichen (compare *Collema*, pages 117-118).

4. The **rhizines,** which may be seen as irregular brushes of colourless tissue projecting from the lower surface.

Examine the thinnest section on the slide under a high power, and passing successively from the upper to the lower surface, observe—

1. The upper broad band of pseudo-parenchyma, the outer limiting part of which is coloured yellow: the cell-walls of this tissue are swollen, and the cell-contents not voluminous or obvious: this band passes without any clearly marked limit into—

2. The **gonidial layer,** where may be seen, embedded in the rather more lax, colourless, fungal tissue, round cells with definite cell-walls, and green-coloured contents—the **gonidia** or Algal constituent of the Lichen thallus.

3. Passing downwards, the colourless tissue of the thallus becomes more lax, and has a distinctly **hyphal** character, betraying more clearly here its Fungal characters. Note carefully how the

hyphæ come into very close communication with the gonidia.

4. Proceeding to the lower limit of the section, a second band of pseudo-parenchyma is reached, which is colourless, but otherwise similar to the upper.

For comparison with the above type, other Lichens should also be examined : for instance, *Usnea barbata*, which is frequently to be found growing on the bark of trees in hilly districts : it is a **fruticose** Lichen, with a cylindrical, much branched, grey thallus, which bears near the tips of the branches the disk-shaped **apothecia.** Cut transverse sections of the thallus and treat as before : observe—

1. The irregular outline of the nearly circular section.
2. The broad band of external pseudo-parenchyma.
3. The gonidial layer as in *Parmelia.*
4. A broad, and very lax hyphal band.
5. A central dense strand of pseudo-parenchyma with thick swollen walls.

Thus *Usnea* is again an example of a heteromerous Lichen, but of the fruticose type.

Examine the gelatinous thallus of *Collema pulposum*, which is to be found growing on moist soil, stones, &c. The foliaceous thallus is somewhat thick and bulky, and of a gelatinous consistency when moist ; when dry it is relatively thin, and brittle. It is well to observe the changes which moistening produces on dry specimens.

Cut sections through the dry thallus, soak them in water, and mount in water, or in weak glycerine : examine under a high power : it will then be seen that the thallus consists of—

1. A gelatinous transparent matrix, similar to that of *Nostoc* (see page 95).
2. Chaplets of cells coloured greenish-blue (**Algal cells**), occasionally interrupted by larger cells, with thicker walls and no green colour (**heterocysts**).
3. Branched and colourless **Fungal hyphæ.**

Note that the Algal and the Fungal constituents are distributed uniformly throughout the thallus, the Alga not being restricted

to a definite zone: this is thus an example of the **homoömerous** Lichens.

III. Some specimens of *Parmelia* will be found to show more closely packed convolutions than others, and, on these it may be noted that **apothecia** are few, or entirely absent: it is on these that the **soredia** are more especially to be sought for. Soak such a specimen (which has previously been kept dry) in water: then, having dried off the excess of water with blotting-paper, press its upper surface on a glass slide, when, on removing it again, a sediment will be left in the water on the slide: mount this in a drop of glycerine, warm gently, and examine under a high power. Various objects which have no relation to the thallus will be seen, such as grits, various Algæ, &c. &c. Amongst these will be seen roundish bodies (**soredia**) of various size and complexity, composed of the same constituents as the thallus, viz.—

a. Fungal hyphæ, enveloping, and completely inclosing—

b. The gonidia, of which one or more may be present in each soredium.

Attempts should be made to grow Lichens from the soredia: pieces of porous tile should be heated to kill other organisms, then saturated with water, and the soredia sown in small numbers on their surface.

In some other Lichens the soredia are produced in a more prominent manner than in *Parmelia:* thus in *Usnea* and *Cladonia* they may be recognised as a powdery covering of some parts of the thallus, and are especially obvious after rain. In the gelatinous Lichens soredia are absent, but the thallus may be reproduced by the outgrowth and ultimate abstriction of processes consisting of both Algal and Fungal constituents: these may often be seen projecting from the outer surface in sections of these Lichens.

IV. Having carefully noted the form of the apothecium of *Parmelia*, and its attachment to the thallus, cut thin vertical sections through it, and, mounting in glycerine without previously allowing them to swell in water, observe, under a low power—

1. That the structure of the part of the thallus which bears the apothecia is similar to that described above.

2. That the lower stratum of the tissue composing the cup is similar to the above, but note especially the very considerable masses of gonidia immediately below the upper stratum of the apothecium.

3. That the upper stratum of the apothecium consists of closely packed, more or less club-shaped elements, regularly arranged perpendicularly to the surface: this is the **hymenium,** of which two constituents are to be distinguished—

a. The **asci,** which may be recognised as relatively wide, club-shaped cells; the contents vary according to the stage of development: when mature each contains eight oval **spores.** Note that various stages of development are to be seen in the same apothecium.

b. The **paraphyses,** which are relatively narrow, and take no direct part in reproduction.

Treat a fresh section through an apothecium with iodine solution, and note that the cell-walls of the hymenium assume a **blue coloration.**

Treat some sections through an apothecium with water, and observe how greatly the hymenium swells, so that it is thrown into numerous folds: it may be remembered that this property of swelling of the constituents of the hymenium is connected with the rupture, and extrusion of the spores when mature; since the

margin of the apothecium (the **excipulum**), consisting of less swelling tissue, resists the increase of bulk of the hymenium, and pressure is thus established.

Note that the ends of the paraphyses and of the older asci have a yellowish-brown colour, similar to that of the surface of the rest of the thallus: treat sections with potash solution and warm: the parts coloured yellow assume a pink colour, which diffuses out into the solution, and also into other tissues of the thallus: neutralise with acetic acid, the colour will disappear, but may be again produced by adding potash. Compare with this the well-known properties of **litmus**, which is produced from *Roccella tinctoria* and other Lichens.

V. Transverse sections may also be cut through the hymenium: treat as before, and note the **asci**, which appear of circular outline, of relatively large size, and contain the highly refractive spores: the **paraphyses**, of relatively small bulk, closely packed round them.

VI. Observe the spores in detail: this may be done by inverting the surface of an apothecium (previously kept dry) in a drop of water on a slide, when spores will be ejected, by means of the pressure due to swelling as above noted: mount these in water, and examine them under a high power: observe their oval form, and the presence of a highly refractive body at either end, the two being connected by a fine strand: stain with an iodine solution, the highly refractive bodies as well as the connecting strand will stain yellowish-brown (protoplasmic body), the rest of the spore is not distinctly stained (cell-wall).

There is considerable variety in the number of the spores produced in each ascus in various Lichens, and they attain in some cases great complexity of structure, as well as considerable size.

Compare *Megalospora*, where in each ascus only one large

unicellular spore is formed; *Pertusaria*, with two, four, or six in different species; *Peltigera*, *Graphis*, and *Collema*, in which each spore is composed of four or more cells (**sporæ compositæ**).

VII. The discovery of the sexuality in certain of the Lichens is one of the most marked observations of recent years, and attempts should be made to see at least the more important organs concerned in the process, which are on the one hand the **spermogonia** (male), on the other the **ascogones** (female): these may both be seen in species of *Collema* collected in early spring; while the spermogonia, which in other Lichens are often difficult to find, may be observed in any specimen of "Iceland Moss" (*Cetraria Islandica*) as sold in druggists' shops.

Observe on specimens of *Cetraria* a fine fringe of minute teeth along the margin, each of these contains a **spermogonium** at its apex: embed a portion of a thallus bearing these teeth, and cut fine transverse sections of it: the teeth will thus be cut longitudinally. Mount in glycerine, having previously allowed the sections to swell in water, and look over the sections under a low power to find examples of spermogonia cut longitudinally, which will appear as flask-like cavities. Having found one of these, examine it under a high power, and observe—

1. The general structure of the thallus corresponding to the **heteromerous** type above described (*Parmelia*).

2. The hollow **spermogonium,** composed exclusively of the Fungal constituent of the Lichen, the hyphæ being closely packed and pointing radially inwards to the cavity, and giving off at their apices by abstriction the

oval, motionless **spermatia,** which escape by a fine **ostiole.**

The escape of the spermatia may be observed in fresh material, by mounting a piece of the thallus bearing spermogonia in a drop of water, after it has previously been kept dry for a time: the spermatia are then extruded through the ostiole, embedded in a gelatinous matrix. (Compare these spermogonia with those of *Puccinia.*)

VIII. Though the search for the female sexual organs which precede the formation of the apothecia has been successful only in few cases, still there is no great difficulty in observing them in the genus *Collema.* Material for this purpose should be collected in early spring (March), and sections may be cut from it fresh, or better after hardening in alcohol. Cut fine sections from a part of a thallus which bears as yet no apothecia, let them swell in water, or weak glycerine, and mount in glycerine: observe here and there a fungal filament to have become coiled at some distance below the surface, and then to be continued almost directly to the outer surface of the thallus, beyond which it projects—this coiled part is the **ascogone,** while the straight part is the **trichogyne.** Occasionally spermatia are to be seen attached to the apex of the trichogyne.

In other sections apothecia may be seen in various stages of development, or spermogonia having a structure similar to that above described for *Cetraria.*

C. *PYRENOMYCETES.*

CLAVICEPS PURPUREA (Ergot).

I. This Fungus is found infesting the ears of various Grasses : it is very prevalent on the Rye, and is commonly styled the Ergot of Rye ; but it is also to be seen on other Grasses, *e.g.* on *Lolium perenne*, *Glyceria fluitans*, &c.

Examine specimens of ergotised Grasses taken in the dormant condition in autumn or winter: the arrangement of the parts will be found to be normal, but often, in place of the normal ovary, there may be seen an enlarged, hard, dark-purple body, which is easily detached : this is the **sclerotium** of the Fungus. Note at the apex of the sclerotium a lighter coloured, easily detached body : this is all that remains of the **gonidiophore** (Sphacelia form) which is produced in early summer, and is now dry and shrivelled.

II. Cut transverse sections of the sclerotium in the dormant winter condition : mount in glycerine, and observe under a low power—

1. The irregular outline of the section, and compare this with the whole sclerotium, which is often marked by longitudinal grooves or slits.
2. The external dark-purple covering.
3. The internal, dense white mass.

Examine a fine section closely, under a high power: it will then be seen that the whole is composed of a **pseudo-parenchymatous tissue,** with relatively thick cell-walls, and abundant oil stored

in the cells: this collects in large globules on and about the sections. It will be further observed that the peripheral tissue is similar to the central, with the exception of colour and consistency. Small masses of tissue of the ovary of the Grass may sometimes be found embedded in the sclerotium.

Treat a section with Schulze's solution: the cell-walls will stain a faint yellow, and this colour is not changed even after some hours' treatment with the reagent (fungal cellulose).

III. Set sclerotia to germinate, half buried in moist clean sand, at a moderate temperature: this will succeed best in spring or early summer, since even if the cultures be started at other seasons, under most favourable conditions, the sclerotia will (with very few exceptions) undergo no change till the proper season comes round. When germination begins, the peripheral tissue will be broken through at one or more points by the swelling of a light-coloured mass within: this grows quickly, so as to form a **stroma**, which consists when mature of an erect cylindrical **stalk** one inch or less in length, and a spherical **head.** Examine the latter with a lens: it is when mature of a purplish colour, and marked with numerous projecting dots.

IV. Cut transverse sections of a sclerotium which has already germinated, and in such a way as to traverse the base of a mature stroma: mount in glycerine, and observe—

1. That the store of oil, &c., in the sclerotium is much reduced, and the whole tissue soft and apparently exhausted.

2. That the tissue of the stalk is of a distinctly hyphal character, the hyphæ being arranged parallel one to another, and septate.

3. That at the base of the stalk the hyphæ appear to have originated from the pseudo-parenchyma of the sclerotium.

V. Cut median longitudinal sections of the mature head of a stroma, and including the upper part of the stalk: mount as before, and examine under a low power: observe—

1. The upper part of the stalk showing the same structure as the base.

2. The semi-lunar section of the head, inserted upon it.

3. The numerous flask-shaped **perithecia** embedded in its mass, each having a slight papillose projection of the surface opposite it: these have already been observed from outside.

Examine the sections under a high power, especially the head, and note—

1. The darker, denser zone immediately above the insertion on the stalk.

2. The more spongy mass of tissue forming the bulk of the head: in this, the hyphæ are more laxly arranged, and thicker: externally will be seen—

3. A more dense, pseudo-parenchymatous peripheral band.

4. The **perithecia** will now be seen to be cavities, each with an **ostiole** which opens at the apex of one of the papillose projections. Note the rather denser coat of tissue lining the cavity, which widens at the base into a **sub-hymenial** mass: this gives off upwards numerous club-shaped **asci**, closely packed, and without paraphyses; these, when mature, contain the peculiar elongated filamentous **spores.**

VI. Attempts should be made, by cultivation of these spores in water, to observe the first stages of germination, when it may be seen that hyphal tubes are formed at a number of points on the single spore.

VII. Attempts should also be made in early summer to infect the inflorescence of various Grasses on which the parasite is known to grow with the fresh spores, and to follow out the development and characters of the **gonidiophore** or **Sphacelia** form of the Fungus.

D. *CLEISTOCARPOUS ASCOMYCETES.*

EUROTIUM ASPERGILLUS GLAUCUS.

I. Keep a slice of dry bread under a bell-glass, until it becomes mouldy. Even a superficial examination of it will show in most cases that more than one kind of Mould is present. Among the rest the most prominent will probably be one which bears roundish, white or pale green heads closely aggregated, and borne on stalks of about one-sixteenth of an inch in length: this is the conidial form of *Eurotium Aspergillus glaucus*, and the branches bearing the heads are styled the **conidiophores.**

Shake some of these gently with the point of a needle: numerous minute powdery bodies (the **conidia**) will be liberated, and will float away as a fine cloud.

If it be desired to obtain a pure culture of this fungus for further study, the following precautions are to be taken. Thoroughly boil a few French plums till they are quite soft: this will completely sterilise them. Having previously sterilised a plate and bell-glass by exposure to high temperature (boiling-point for a long time, or a higher temperature for a shorter time),

place the plums on the plate, and infect them by transferring a **few** conidia from as pure a patch of the mould as can be found on the bread : the transfer is to be made with a needle which has previously been heated. If these precautions are taken, and the plums be kept covered, a pure culture of the Fungus should be obtained.

II. From a pure patch of this green Mould remove a small portion with a needle, avoiding mechanical roughness as much as possible : lay it on a slide, moisten with a single drop of alcohol, then add water, and cover gently with a cover-slip. Examine it under a low power, and observe—

1. The stalked **conidiophores,** with large, mop-like heads.

2. The colourless tangled **mycelium** attached to these, and from which they spring.

3. The innumerable detached **conidia** which will be found thickly distributed throughout the preparation.

Having selected one of the largest of the conidiophores, examine it in detail under a high power, noting especially—

1. The robust **stalk,** usually without septa: its wall is clearly defined, and the protoplasmic contents granular and vacuolated.

2. The transversely septate, branched **mycelium,** from which the conidiophores arise as vertically growing branches, usually from a point immediately behind one of the septa: in this as in other cases of branching of the mycelium, the branch grows out at right angles from the hypha which bears it.

3. The swollen spherical **head** of the conidiophore, with its conidia in radiating rows inserted upon it.

Examine carefully the way in which the conidia are produced, noting—

a. The **sterigmata,** peg-like radiating outgrowths from the head of the conidiophore.

b. The series of **conidia,** in successive stages of development, which have been successively formed by **abstriction** from the sterigmata.

c. The oval form, and spiny surface of the mature conidium.

In order to observe the successive stages of development of the conidiophore, small portions of the Fungus should be taken from the white patches, where the growth is younger, and be treated as before. In these specimens the following points are to be observed—

1. The conidiophore as a club-shaped thick erect hypha.

2. The swelling of the head, though it remains quite smooth.

3. Minute papillar outgrowths appear on the surface of the head—these are the young sterigmata.

4. The sterigmata elongate, and become attenuated at the tips.

5. The successive stages of abstriction of the conidia from the apices of the sterigmata.

III. In order to trace the germination of the conidia, they should be cultivated under microscopic observation on the slide. For this purpose a moist chamber is to be prepared as directed in Part I., p. 16. It will be necessary to take certain precautions to reduce the probability of access of foreign spores to a minimum, and so insure as nearly as possible a pure culture. Prepare a nutritive solution by boiling French plums in water: this

decoction is to be used **very dilute,** and is to be boiled **immediately before starting the culture,** so as to kill any foreign spores which may be already present: with the same object, the glass slide, cover-slip, and needles are all to be heated in a spirit-lamp, and the porous pad for the moist chamber is to be well boiled in water.

Having made these preparations, place a single drop of the dilute, sterilised decoction on the cover-slip: then with a needle, moistened with the sterilised fluid, remove from as pure a tuft of *Eurotium* as can be found a **small number** of conidia, and place them in the single drop on the cover-slip: examine under a low power to see that the number of conidia is small, then quickly invert the cover-slip and place it over the round hole punched in the porous pad. Keep the preparation thus made under a bell-glass, and observe it from time to time under the microscope: if the culture be successful, the successive stages of germination and of further development of the Mould may be watched in detail.

IV. The **perithecia,** and the **archicarps** (female organs) which give rise to them, are to be sought for on a mycelium which has already produced mature conidia: the ripe perithecia (*Eurotium* fruits) may be readily recognised in old cultures on dry bread, as minute yellowish spherical bodies, easily distinguished by the naked eye.

A. Remove a small piece of mycelium which has already borne mature conidiophores, and is thus likely to bear young **archicarps:** moisten it with alcohol, and then wash off in a watch-glass in water as many of the conidia as possible: tease it out with needles,

and, mounting in water, examine under a high power. Observe—

1. That the same mycelium which bears the conidiophores also produces relatively thin whip-like branches, with highly refractive contents.

2. That some of these branches become coiled, at first loosely, but later in a tightly packed spiral of four or five coils, and consisting of several cells: these spirals are the **archicarps.**

3. That first one, and subsequently several hyphal branches appear below the closely coiled archicarp, forming an investment round it: the first formed branch is called the **pollinodium** (male organ), and comes in close contact with the apex of the coiled archicarp.

The actual transmission of substance from the pollinodium to the archicarp has not been seen, but observations point to the disappearance of the membrane separating them, and thus continuity appears to be established between the two protoplasmic bodies.

B. From a culture of some six weeks' duration on dry bread pick off with a needle some of the minute spherical perithecia: mount them in water, and examine under a low power: observe—

1. The round or oval form of the perithecia.

2. That they are composed of a small-celled pseudo-parenchymatous tissue.

3. Their yellow colour.

4. Their insertion, each being borne on a single filament of mycelium.

The yellow colour is due to an oily substance, which is soluble in alcohol, or in potash solution.

Treat some perithecia with a weak potash solution, mount them in glycerine, and examine under a high power: note—

1. The **wall** of the perithecium, consisting of a single layer of somewhat flattened cells.

2. The cavity surrounded by that wall filled with bodies of oval form—the **asci.**

In order to be able to examine the asci in detail, mount fresh perithecia in glycerine, press with a needle on the cover-slip, so as to burst them, and note—

1. The ruptured wall, as before.

2. The oval **asci,** each of which contains eight **asco-spores,** of oval shape when young, and biconvex-lens shaped when mature.

3. Other cells may also be found which belong to the filling-tissue or pseudo-parenchyma; this is derived by ingrowth from the wall of the perithecium, and is only to be found in young perithecia: at the period of maturity it is completely absorbed.

In order to trace the various steps of development of the perithecium, observations should be made at various times during the progress of the culture, and the origin of the asci from the spirally coiled archicarp is to be observed in specimens made transparent with potash and glycerine. Further, the origin of the wall of the perithecium from branch filaments which grow round and invest the archicarp is also to be traced, and finally the ingrowth of the wall between the products of the archicarp, so as to form the "filling-tissue."

These points, however, and especially those changes which take place in the later stages of development, are best to be seen in sections cut through the perithecium: these may be prepared by carefully embedding in paraffin, or, better, by embedding in white of egg (see Part I. page 5): first moisten with alcohol, and

then wash well with water, and soak thoroughly in the white of egg: coagulate it, and harden in alcohol. Sections are then to be cut of the whole mass, together with the bodies embedded, and they are to be mounted and examined in the usual way.

Attempts should also be made to cultivate the mature spores in a very weak decoction of French plums as above directed for the conidia.

IV. PERONOSPOREÆ.

PYTHIUM DE BARYANUM.

I. Sow seeds of the common garden Cress (*Lepidium sativum*) thickly in a flower-pot: cover it over with a glass plate, and keep it well watered, so that the young seedlings grow up in an atmosphere saturated with water. After a few days the heads of some of the seedlings may be seen to have bent over, owing to insufficient support of the stem: examination will show that the curvature is a sharp one, so that the curvature is not due to general weakness: further that the stem is thin and flabby at the point of curvature: while fungal filaments may be observed in close contact with the stem at that point, and it is this Fungus (*Pythium de Baryanum*) which is the cause of the disease termed by gardeners "damping off": it is of common occurrence in propagating pits which are kept too warm and moist.

Other members of this, and other allied genera may also be present, but the species above named is almost certain to appear on damp cultures of the common Cress: the difficulty of distinguishing these species from one another may cause apparent discrepancy between the observations and the description given below.

If the Cress cultures be kept damp for some days longer, a thick felt of hyphæ will be formed, which will bind the seedlings together: and finally the disorganisation, which usually begins near the base of the hypocotyledonary stem, will spread throughout the seedlings, causing complete rotting.

II. Mount part of a stem of one of the collapsed seedlings in water, and examine under a low power: observe—

1. That the tissues show an abnormal appearance at the point of curvature, their colour is yellowish, and the individual cells show signs of having lost their turgidity.

2. That numerous colourless branched **hyphæ** extend along the surface of the seedling, being most numerous at the point of curvature, and less frequent further up.

III. Tease out a portion of the infected part, as well as of the healthy part above, with needles in water, and mount so that a part at least of the epidermis shall be seen in external surface view; or sections may be cut, the infected part being held between pieces of pith: in such preparations observe—

1. The healthy part of the epidermis with elongated cells, and occasional stomata.

2. The branched, highly refractive, and for the most part non-septate **hyphæ,** running with an irregular, but mostly longitudinal course along the outer surface.

3. Mark especially the points of entry of the Fungus into the host-plant: this may be either—

a. By **perforation** of the outer wall of a cell of the

epidermis; and this is by far the more common; or—

b. By passage of the hypha **through the pore of a stoma**: this is the less common mode.

4. Trace the further course of the hypha through the transparent tissues of the host-plant, noting the **rarity, or complete absence of septa.**

IV. From diseased specimens hardened in alcohol, cut transverse sections: mount in weak glycerine, together with a drop of iodine solution, and examine under a high power. In these specimens the above observations are to be severally confirmed: it is further to be noted that the hyphal filaments traverse the cell-walls of the host, showing a slight constriction at the point of perforation: also that they traverse the whole epidermis and cortex, either passing directly through the cells, or running along the intercellular spaces.

V. Place an infected seedling in fresh water, in a flat watch-glass, and examine it at intervals for a day or two under a low power. Many of the filaments will be seen to form swellings at certain points, which assume a spherical form, are filled with granular protoplasm and are divided off by a septum from the parent filament, while the thin outer wall assumes a darker colour: these swollen bodies are the asexual reproductive organs, or **resting conidia.** Two types are to be distinguished—

1. **Terminal** conidia, at the ends of the filaments.

2. **Interstitial** conidia, which may appear at any other point on the filament.

It is characteristic of this species that the hypha

should be partially or completely emptied of protoplasm for a short distance below the conidium.

These conidia are capable of withstanding drought, or a temperature below freezing, without losing their vitality.

VI. From a culture containing numerous conidia separate a small portion, and expose it in a watch-glass to a relatively considerable bulk of fresh water: examine the culture at intervals under a low power. Some of the conidia will be seen to germinate by the formation of tubular hyphæ similar to those which produced them.

De Bary and Hesse have also described how certain of these swellings, differing in no structural characters from the directly germinating conidia, develop as **zoosporangia**, by the formation of a lateral beak-like outgrowth, into which the protoplasmic contents pass: a division of the protoplasm then takes place, to form numerous **zoospores**, which escape, and after a motile period, settle and germinate as new individuals. This may often be observed in mixed cultures on placing the specimen in a considerable bulk of fresh water.

VII. Continue at intervals the observation of those cultures which have already produced conidia: the formation of the **sexual organs** will frequently be seen to succeed that of the conidia.

a. The **oogonium** resembles at first the conidium in being spherical, and about of equal size with it, and is partitioned off by a septum; a central spherical mass of protoplasm (the **oosphere**) is to be recognised.

b. The **antheridium** arises as a branch, either from the same filament as the oogonium, or from another: its apex is cut off by a septum, and it comes in

close contact with the oogonium: a cylindrical process from it passes through the wall of the oogonium, and gains access to the oosphere.

c. In more mature specimens the oogonium contains a single round, distinctly walled cell (the **oospore**), which lies freely within it.

In order to follow the process of fertilisation, which is to be seen with distinctness in this plant, portions of a well-nourished culture should be cultivated in a damp chamber, in a suspended drop of water: by selecting an oogonium and antheridium of suitable age, the actual transfer of the granular protoplasm from the antheridium to the oosphere may be followed. (See Marshall Ward, Q.J.M.S. xxiii., page 490, &c.)

Cystopus candidus (White Rust of Shepherd's Purse).

Observations on *Cystopus* are so easily made that they should not be omitted. The Fungus is commonly to be found growing parasitically upon *Capsella Bursa-pastoris*, and others of the *Cruciferæ*, causing in summer white eruptions, accompanied by swelling, and greater or less malformation of the vegetative organs, and even of the flower and fruit. It must not be confounded with the equally common *Peronospora parasitica*, which is to be found at similar times on similar plants, and bears a superficial resemblance to it.

Note with a lens the white patches: some will be seen to be still completely covered by the epidermis, in other cases rupture will have taken place, and the mealy mass of white **conidia** is thus exposed.

Cut longitudinal sections of the stem of *Capsella* so as to traverse one of the blotches, and, examining under a high power, observe—

1. The general arrangement of tissues as in a typical Dicotyledon.

2. The colourless non-septate **hyphal filaments,** which traverse the intercellular spaces, not only of the cortical parenchyma, but also of the pith : note carefully the button-like **haustoria,** which the Fungus puts out through the cell-walls into the cavities of the cells of the host.

3. Where the section has traversed the white blotch, the epidermis is separated from the subjacent tissue by a mass of hyphal filaments, each of which terminates in a chain of **conidia,** formed successively by abstriction.

4. Frequently, and especially in material taken in autumn, the sexual organs of the Fungus are to be seen borne on hyphal filaments within the tissue of the host. The main characters of the **antheridium** and **oogonium** correspond to those of *Pythium:* when ripe the **oospore** is covered by a dark-coloured and rugose **exospore,** as well as by the colourless inner layers (**epispore** and **endospore**).

Attempts should be made, by placing fresh conidia in a drop of water on a slide, to observe their germination : the contents divide into a number of parts, which escape as motile **zoospores.** Further, the behaviour of these during germination on a fresh leaf of *Capsella*, and the entry of the germinal tubes through the pores of the stomata are to be observed.

Oospores are also to be dissected out from the tissues of the host plant in spring (*i.e.* after the period of rest), and to be cultivated in fresh water : their germination is to be observed, the protoplasm dividing into a large number of swarm-spores, which escape and develop further in the same way as the zoogonidia.

Observations may also be made on the Potato Fungus (*Phytophthora infestans*) the mycelium of which permeates the tissues of the Potato plant, while its branched conidiophores project through the stomata.

V. MUCORINEÆ.

MUCOR MUCEDO, *Fres.*

I. If a slice of bread be soaked in water, and kept under a bell-glass, various moulds will make their appearance upon it: about the fourth or fifth day there will be seen a mould, which at first appears white and flocculent, producing long unbranched stalks, which terminate in round heads, white at first, and subsequently becoming black: this will be *Mucor Mucedo.* It may also be obtained on horse-dung kept under a bell-glass, and on various other substrata.

II. Remove a very small piece of the bread bearing the mould, and tease it out gently in water: mount and examine under a low power: note—

1. Relatively thick, non-septate hyphæ, which ramify in the substance of the bread.

2. Relatively thin branches, which are produced from the thick ones, and themselves, branching repeatedly, produce a very extensive system of minute fibrils.

3. Hyphæ similar to (1), which however grow erect in the air (**gonidiophores**), each bearing at its summit one spherical **sporangium**: this will certainly have

been damaged in the process of preparation. For the observations on its structure see below.

By hardening in alcohol, or by fixing with picric acid, and subsequently treating with staining reagents, it may be demonstrated that numerous nuclei are present in the hyphæ of *Mucor*.

It has been stated above that the hyphæ of *Mucor* are not septate: this is the case in young cultures, but preparations should also be made from old cultures, after the production of the sporangia: in these numerous septa may be found.

It has also been stated that the gonidiophores are unbranched: that is so in the large majority of cases, but in old cultures lateral branches may be found arising below the sporangium, and themselves terminated in turn by a sporangium.

III. Cut off a number of mature sporangia with scissors from the flocculent growth, treating them very gently, so as to avoid damage: mount them in alcohol, and examine them quickly under a low power: observe—

1. The cylindrical **gonidiophores,** terminated by—

2. The spherical and dark-coloured **sporangium,** with its dense contents, and its very thin limiting wall, often bearing small radiating projections.

3. Towards the point of attachment to the stalk a clearer space may be recognised in the contents; this indicates the position of the **columella.**

Add a drop of water, and draw it under the cover-slip with blotting-paper, watching the effect upon the sporangia: as the water gains access to the sporangia, they burst suddenly, and the wall may be torn to fragments so minute that it cannot be recognised again. Meanwhile the contents, the swelling of which caused

the rupture, gradually distend, and may be recognised as consisting of—

4. Numerous oval **spores,** with smooth walls.

5. An intermediate **mucilaginous substance** which is capable of swelling, and thus effects not only the bursting of the sporangium, but also the dispersal of the spores.

6. After the swelling and dispersal of the spores are complete, there will be seen remaining a spheroidal body (the **columella**), which is the distended septum of separation of the sporangium from the gonidiophore: round its base may often be traced the remains of the wall of the sporangium as a ragged fringe.

The minute projecting bodies on the surface of the sporangium are not of constant occurrence, though often present: they consist of oxalate of lime.

According to the conditions of nutrition of the Fungus, there may be very considerable variety in the size of the sporangia, and in the number of the spores produced: under peculiar circumstances the sporangia may be of so reduced a form that the columella is absent, and the number of spores may be less than ten.

IV. With similar precautions to those taken in the case of the spores of *Eurotium Aspergillus* (page 128), sow spores of *Mucor* in a drop of a sterilised decoction of horse-dung, or of French plums, or other suitable solution: the swelling and germination of the spores, and the formation of the branched, non-septate mycelium are to be watched; and drawings may with advantage be made at intervals, so as to record the progress of the cultures.

The *Torula* condition may be induced in certain of the species of *Mucor*, especially in *M. racemosus* (though not so readily in *M. mucedo*), by growing the mycelium immersed in a nutritive solution such as Pasteur's solution: the hyphæ then become partitioned off by transverse septa into short **gemmæ.** During this stage alcoholic fermentation may be effected by it. On being re-exposed to the air, under other suitable conditions, the gemmæ may germinate and produce a normal mycelium. Observations should be made on these points.

SPORODINIA GRANDIS, *Link.*

V. *Mucor mucedo* also reproduces itself by means of **zygospores,** which are of such size that they may be detected with the naked eye as black bodies which project slightly from the substratum; but they are not of constant occurrence, and may frequently be looked for in vain. Accordingly it will be found more convenient and successful to study the development and structure of the zygospores in an allied form, in which they are produced in profusion, viz. in *Sporodinia grandis*, Link. (= *Syzygites megalocarpus*, Ehr.).

Sporodinia is a fungus which may commonly be found in autumn, growing parasitically on many of the larger, fleshy Hymenomycetes, especially on *Russula*, or *Boletus*: it appears as a greyish or brown flocculent growth, and the zygospores are of such a size that they can readily be seen as reddish-brown bodies with the naked eye. While a part of the mycelium ramifies in the tissue of the host, the zygospores are borne on aërial branches: they may thus be easily recognised as brown bodies, visible to the naked eye.

Tease out a small piece of the flocculent mycelium

gently in water: examine under a low power, and observe—

1. The branched **hyphæ,** which are light-coloured, and rarely septate when young, but assume a brown colour, and form numerous transverse septa at irregular intervals as they grow old.

2. The large brown **zygospores,** each supported by two thicker, club-shaped hyphæ (*Syzygites* form).

3. The relatively small **sporangia** borne on branched gonidiophores, and having a structure similar to those of *Mucor* (*Sporodinia* form).

Compare a number of zygospores in various stages of development, and observe in them the following points—

1. The swelling of two neighbouring mycelial filaments (**suspensors**), and their assumption of a position with their two swollen ends opposite one another.

2. The formation of transverse **septa** cutting off the apical part of each suspensor, thus forming the two **gametes.**

3. The two **gametes** in close contact with one another, while the walls at the point of contact are gradually absorbed, the absorption beginning at the central point: the two protoplasmic bodies thus coalesce to form the **zygote** or **zygospore.**

4. The increase in size of the zygospore, its contents becoming dense and oily, while the wall at the period of maturity consists of the following successive layers—

a. The **primary membrane** of the gametes, which remains thin, but persistent as an external covering.

b. The **epispore,** which is a dark-coloured firm or

brittle layer with hemispherical wart-like outgrowths from the surface.

c. The **endospore,** which is thicker and more transparent.

Note how numerous though irregular are the septa in mycelium which has produced zygospores.

It is not an uncommon thing in *Sporodinia* to find that the two gametes may not come in contact, and no zygote be found; but still each gamete may develop into a body resembling a zygospore in the character of the wall, the contents, and in the mode of germination. These bodies are called **azygospores,** and the phenomenon may be regarded as a form of apogamy.

Attempts should be made to germinate the zygospores. This may be done by keeping them under observation in fresh water for some weeks during the autumn, changing the water frequently: the brown epispore ruptures, the endospore protrudes, and forms two to four germinal filaments. If such germinating spores be now cultivated on a moist substratum, the filaments may form gonidiophores of the *Sporodinia* type.

THE END.

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